The α^2 HS glycoprotein receptor on lymphocytes transformed by Epstein–Barr virus

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Received 4 May 1982

The α_2HS glycoprotein receptor form lymphocytes transformed by Epstein–Barr Virus was isolated by affinity chromatography. The protein receptor has a monomer M_r of 48 000, which is similar to the Epstein–Barr virus-determined nuclear antigen (EBNA), and pI = 7.2. Like EBNA the 48 000 M_r component in unfractionated labelled detergent solubilised cell supernatants also binds DNA. These results may suggest some similarity between the α_2HS receptor and EBNA.

α_2HS glycoprotein *Epstein–Barr virus* *Virus transformation* *Glycoprotein receptor*

1. INTRODUCTION

Epstein–Barr Virus (EBV) infects B lymphocytes leading to immortalization of the cell line [1]. Cells transformed by EBV synthesize and express sequential sets of virally-determined antigens. The initial expression of the EBV-determined nuclear antigen (EBNA) is closely followed by the virus-induced, lymphocyte-detected membrane antigen (LYDMA) [2]. Expression of other viral-determined antigens is tightly controlled and dependent on the induction of a productive or lytic cycle which leads to the synthesis of early antigen (EA), viral capsid antigen (VCA), and late membrane antigen (LMA) [1]. Isolation and characterization of EBV-determined antigens is important for understanding the control of EBV infection and attention has been focused on EBNA and LYDMA which are expressed in non-productive infection [1], and may play a role in immune defense mechanisms against EBV infection [3].

We had supposed that the serum protein α_2HS glycoprotein (α_2HS) could fulfil criteria as a mediator enhancing host defense mechanisms against EBV-transformed cells, as it binds to lymphocytes transformed by EBV [4], promotes macrophage phagocytic function [5,6] and acts as an opsonin during bacterial phagocytosis by human neutrophils [7]. The ability of α_2HS to bind to lymphocytes transformed by EBV, but not to normal autologous non-transformed lymphocytes [4], therefore necessitates the isolation and characterization of the α_2HS receptor.

Here we report the isolation of the α_2HS receptor from labelled detergent solubilized EBV-transformed lymphocyte supernatants by affinity chromatography. The protein receptor has a monomeric M_r of 48 000, which is similar to EBNA, and pI = 7.2. Like EBNA, the 48 000 M_r component in unfractionated, labelled detergent solubilized cell supernatants also binds to DNA. These results may suggest some relationship between the α_2HS receptor and EBNA.

2. MATERIALS AND METHODS

Peripheral blood lymphocytes were transformed with EBV culture supernatants derived from the B95-8 cell line [8]. The mycoplasma and bacteria free cell line was cultured as in [4]. Transformed cells ($\sim 10^7$) were washed 3× with phosphate-buffered saline (PBS) and ^{125}I-labelled by the peroxidase method [9]. The labelled cells were detergent-solubilized with 1.0 ml borate-buffered saline (BBS) (pH 8.5) containing 1% NP40 and 2 mM phenylmethylsulphonyl fluoride (PMSF) and the 30 000 × g supernatant incubated with 1.0 ml α_2HS–Sepharose 4B (2.5 mg α_2HS/ml Sepharose

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

4B) overnight at 4°C. Unbound material was removed by washing the column with 100 ml BBS containing 1% NP40. Bound material was eluted at 4°C with 0.5 M acetic acid containing 1% NP40. Following dialysis the bound fraction was concentrated by ultrafiltration. Purified α_2HS receptor and labelled, unfractionated, detergent-solubilized cell supernatant (10 μl) were also subjected to pronase or RNase digestion (2 mg/ml in PBS containing 2 mM $MgCl_2$) for 60 min at 22°C.

Gel filtration was carried out in a 2.5 × 90 cm column using Aca 34. Fractions, 30 min, were collected at 27 ml/h. Columns were run in BBS containing 1% NP40. ^{125}I-α_1AT and ^{125}I-CEA were used as M_r-markers. Fractions were counted to determine elution profiles.

Isoelectric focusing was carried out in 4% polyacrylamide disc gels in the presence of 8 M urea and 4% ampholine solution, pH 3–10 (Sigma).

Samples were loaded at the cathode and haemoglobin used to monitor focusing. At completion gels were sliced into 2 mm segments and placed in tubes along with 0.5 ml deionised distilled water for elution. Gel slices were counted and the pH of the eluted fractions determined.

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out according to Laemmli [10] in 5–20% gradient slab gels. Following electrophoresis gels were fixed in 10% trichloroacetic acid, stained, destained and prepared for autoradiography by drying onto Whatman 3MM paper. M_r-Markers were phosphorylase B (97 000), bovine serum albumin (68 000), ovalbumin (44 000), carbonic anhydrase (29 000), and lysozyme (14 000). ^{125}I-α_1AT (52 000) was used as a marker for autoradiography. Samples were incubated with 2% SDS, 95°C for 5 min, prior to electrophoresis, usually in the presence of mercaptoethanol.

DNA–agarose affinity chromatography was carried out on the α_2HS receptor and labelled unfractionated detergent solubilized cell supernatants in 10 mM phosphate buffer (pH 6.6). Following binding, 4 h at 22°C, the bound fraction was eluted with 10 mM phosphate buffer (pH 6.6) containing 0.5 M NaCl and concentrated by ultrafiltration.

3. RESULTS

The isolation procedure resulted in the elution of ~0.01–0.1% of the radioactivity applied to the α_2HS–Sepharose 4B column. The bound radioactivity was routinely eluted with 0.5 M acetic acid containing 1% NP40 which was equally effective as competitive elution with α_2HS solution (2 ml–2.0 mg/ml in PBS with 1% NP40) (fig.1). Following dialysis the acid-eluted receptor suffered an 80% loss in rebinding to the affinity column and did not bind to DNA–agarose. Gel filtration of the ^{125}I-α_2HS receptor along with ^{125}I-α_1AT is shown in fig.2. The ^{125}I-α_2HS receptor like ^{125}I-CEA

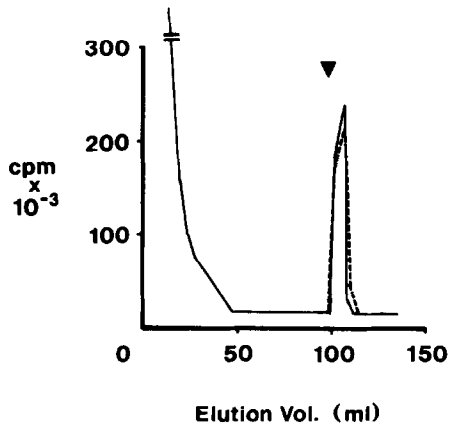

Fig.1. Affinity purification of receptor on α_2HS–Sepharose-4B. The column was washed with BBS containing 1% NP40 and the bound fraction eluted, (▾). Elution profile is shown using α_2HS solution (——) or acetic acid (---).

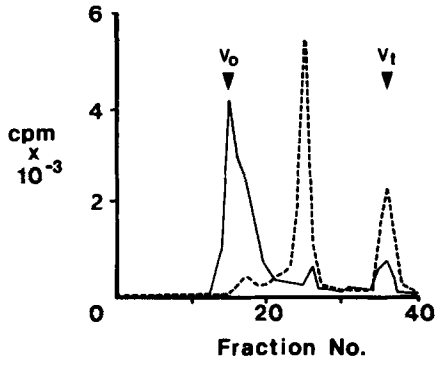

Fig.2. Aca 34 gel filtration of α_2HS receptor (——) and ^{125}I-α_1AT (---). The void and column volumes are indicated as V_0 and V_t, respectively.

(200 000 M_r) eluted at the void volume (V_o), ^{125}I-CEA elution profile is not shown. Isoelectric focusing of the α_2HS receptor revealed a pI = 7.2±0.1 (n = 3) with some material barely penetrating the polyacrylamide gel (fig.3). SDS–PAGE results are shown in fig.4. Autoradiographs of the α_2HS receptor revealed a monomer M_r = 48 000 in both the presence and absence of mercaptoethanol. An aggregated form showed at the origin. The 48 000 M_r band was also present in labelled, unfractionated, detergent-solubilized cell supernatants in both the presence and absence of mercaptoethanol. An autoradiograph of RNase-treated, labelled, unfractionated, detergent-solubilized cell supernatants is also shown. Pronase treatment of the α_2HS receptor and labelled, unfractionated, detergent-solubilized cell supernatants abluted all autoradiographic bands whereas treatment with RNase did not. Autoradiographs of the labelled, unfractionated, detergent-solubilized cell

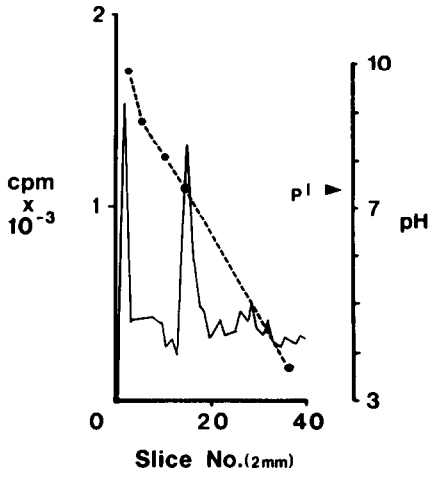

Fig.3. Isoelectric focusing of α_2HS receptor in 4% polyacrylamide gels. Following focusing gels were sliced and counted (——). The pH gradient is also indicated (•——•). The isolectric point is shown (►) (pI = 7.2).

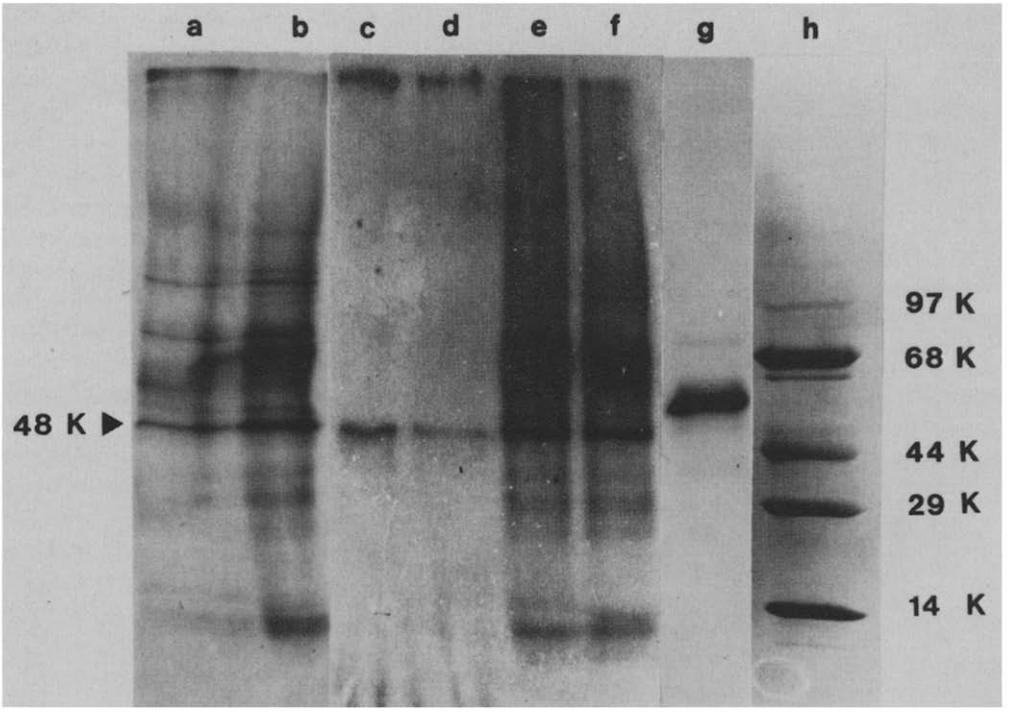

Fig.4. Autoradiograph of ^{125}I-labelled proteins analyzed by SDS–PAGE. Labelled unfractionated detergent solubilized EBV-transformed lymphocyte supernatants without mercaptoethanol (a); with mercaptoethanol (b,e); with mercaptoethanol after RNase treatment (f). α_2HS receptor in the presence and absence of mercaptoethanol, (c) and (d), respectively. ^{125}I-α_1AT is shown, (g); as are protein standards stained with Coomassie blue (h). The 48 000 M_r receptor band is indicated.

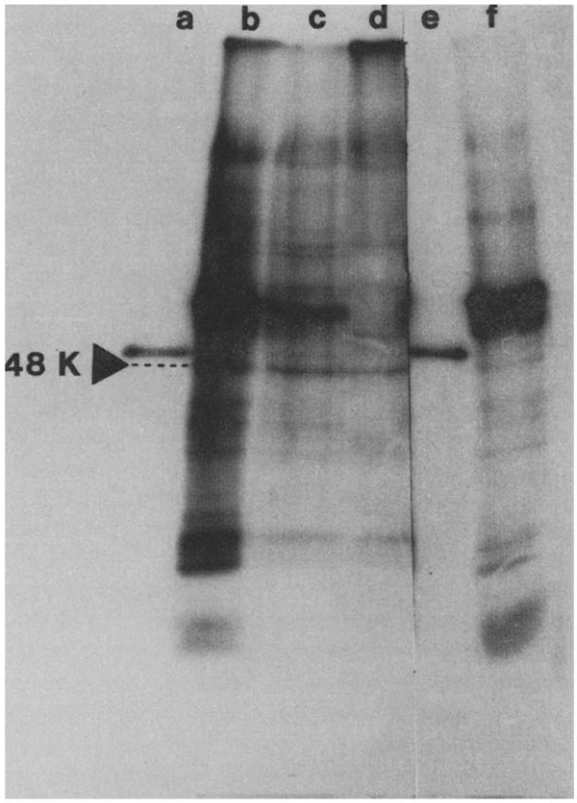

Fig.5. Autoradiograph of labelled, detergent-solubilized cell supernatant DNA-binding proteins from EBV-transformed lymphocytes. ^{125}I-α_1AT (a, e); labelled, unfractionated, detergent-solubilized cell supernatant proteins reduced with mercaptoethanol (b); DNA–agarose bound proteins in the presence and absence of mercaptoethanol (c) and (d), respectively. Proteins not retained by DNA–agarose incubated with mercaptoethanol (f). The 48 000 M_r band is shown.

supernatant and the DNA-binding fraction are shown in fig.5. The 48 000 M_r band was present in both the unfractionated and the DNA-binding samples, again under both reducing and non-reducing conditions.

4. DISCUSSION

Transformation of B lymphocytes with B95-8 supernatants results in non-productive infection and as such EA, VCA and LMA would not be expected to be expressed [1]. Kutner and Sugden have detected a unique antigen on the cell surface during non-productive infection of 45 000 M_r which may correspond to LYDMA [11]. Like LYDMA, EBNA which is also expressed in non-productive infection has received much attention as it is considered to be an EBV-transforming protein [12], EBNA binds to DNA [13] and M_r determinations vary from a tetramer with a monomeric M_r = 48 000 [13,14]; 50 000 M_r [15]; 65 000 M_r [16] to a complex consisting of a 100 000, 50 000 and a 70 000 M_r heat-labile component [17]. This variation in M_r thought to reflect multiple antigenic determinants in EBNA [16]. The monomer M_r of the α_2HS receptor and its existence as an SDS dissociable, aggregate form is in agreement with that of EBNA [13,14] although its apparent inability to bind DNA may preclude it being this antigen. However, our acid elution technique could conceivably cause loss of this property as an 80% loss in rebinding to α_2HS–Sepharose 4B occurred. It was not possible to test whether the α_2HS receptor with α_2HS solution bound to DNA–agarose as their similar M_r-values would have made separation difficult. Complete separation would have to be achieved as α_2HS itself binds to DNA [18]. The basic pI of the α_2HS receptor, pI = 7.2, makes it a likely candidate for binding to DNA on the basis of charge alone. DNA–agarose affinity chromatography of the labelled, unfractionated, detergent-solubilized cell supernatant revealed a 48 000 M_r DNA-binding protein. The identical M_r of this DNA-binding protein to the α_2HS receptor and their identical migration under both reducing and non-reducing conditions strongly suggests they are the same protein. As the α_2HS receptor is identical in size to the M_r of EBNA reported in [13] and it appears that like EBNA, the α_2HS receptor binds DNA, some relationship between the α_2HS receptor and EBNA is suggested.

The exact relationship of the α_2HS receptor to EBNA should be clarified by the production of specific antibody to the α_2HS receptor and immunological comparison with precipitates produced by anti-EBNA serum.

ACKNOWLEDGEMENTS

The DNA–agarose was kindly provided by Dr A.E. Reeve. We thank the Medical Research Council of New Zealand for support and Dr P.S. Crosier for transforming the cells.

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