Journal of Cellular Biochemistry 30:41–49 (1986)

Antibodies Specific for the Carboxy-Terminal Region of the Major Surface Glycoprotein of Simian Rotavirus (SA11) and Human Rotavirus (Wa)

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Antibodies specific for the major outer capsid protein (VP7) of the simian rotavirus SA11 were obtained by immunization of rabbits with a synthetic peptide, Ser-Ala-Ala-Phe-Tyr-Tyr-Arg-Val, corresponding to the eight carboxy-terminal amino acids of the viral protein predicted from the nucleotide sequence of the gene segment 9 of the SA11 genome. As the carboxy-terminal region of the VP7 of human rotavirus Wa has an identical sequence, cross-reactivity of the raised antibodies was observed with this strain.

Key words: rotaviruses, antipeptide serum, ELISA, immunoprecipitation, cross-reactivity

Rotaviruses are members of the reoviridae family. They are of interest for their ability to cause infectious gastroenteritis in young children and animals (for reviews, see [1–3]).

The simian rotavirus (SA11) has become a common model system for the study of rotaviruses. The genome of the SA11 consists of 11 segments of doublestranded RNA and of a double layered capsid [4]. The inner capsid is composed of the proteins VP1, VP2, and VP6, which could be assigned to the segments 1,2, and 6 [5,6].

The outer capsid is built by VP3 and VP7 coded by segments 4 and 9, respectively [5,7]. The major outer capsid protein VP7 is glycosylated [8]. The serotype specificity is associated with this protein, which is able to elicit the neutralizing antibody response during infection [9]. It is obvious that only a few distinct epitopes can be located on this glycoprotein as shown previously [10].

Several recent studies have demonstrated that antibodies can be raised against chemically synthesized peptides (reviewed in [11]). This very powerful tool has a great advantage in that the raised antibodies should only be able to react with clearly defined epitopes. As the amino acid sequence of the VP7 has been deduced from the

Received April 17, 1985; revised and accepted Spetember 30, 1985.

nucleotide sequence of the gene 9 [12] the possibility of using a synthetic antigen for raising specific antibodies exists. Thus, Gunn et al [13] have raised antibodies against six peptides corresponding to different regions of the SA11 VP7 molecule not including the carboxy terminus. Recognition of the VP7 protein in a blotting experiment was reported. However, none of the antipeptide sera was able to recognize whole virus in a radioimmunoassay (RIA), nor were hyperimmune sera prepared against SA11 able to react with any of these peptides in a RIA.

By means of the Kyte-Doolittle plot [14], it can be shown that the carboxy-terminal region of the VP7 contains a sequence of moderately hydrophatic character. We synthesized the carboxy-terminal octapeptide Ser-Ala-Ala-Phe-Tyr-Tyr-Arg-Val for the following reasons: (1) The carboxy group is hydrophilic by its charge. Antisera against synthetic peptides corresponding to the carboxy termini of polyoma virus medium tumor antigen [15] and SV40 large tumor antigen [16], respectively, have successfully been demonstrated to elicit antibodies capable of precipitating the viral proteins; and (2) the synthesized sequence seems to be conserved in different serotypes [17,18] with the exception of two substitutions in the case of the human rotavirus serotype 2 (Hu/5).

In this report we describe the preparation and the specificity of the antipeptide-serum.

MATERIALS AND METHODS

Synthesis of the Peptide

For this study, the peptide was synthesized by a solid-phase method [19], employing the 9-fluoromethylmethoxycarbonyl (Fmoc) group [20] for amino-protection. The Fmoc-group can be cleaved by mild basic conditions. The side chain protection of Tyr and Ser is achieved by the acid labile tertiary butyl group, whereas Arg is used as the 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) derivative.

The loading of the resin [21,22] with the first amino acid was 0.46 mmol/g. Deprotection was done by the reaction with 50% piperidine in dichloromethane. After several washings, the next amino acid was introduced by coupling the three-fold excess with N,N′-dicyclohexylcarbodiimide and hydroxybenzotriaxole. The reaction was monitored by an qualitative ninhydrin test. Treatment of the peptide resin at the end of the synthesis with 90% trifluoroacetic acid in thioanisole containing 0.2 M methanesulfonic acid gave complete cleavage and removal of the protecting groups.

After purification of the crude product on a reversed phase column, the main product was homogeneous by high performance liquid chromatography and thin-layer chromatography. The composition of the peptide was confirmed by amino acid analysis.

Preparation of the Synthetic Antigen

The peptide was added at a 30-fold M excess to 0.15 μM bovine serum albumin (BSA) in 1 ml of 0.1 M sodium phosphate buffer (pH 7.5) [23]. Three hundred microliters of 21 mM glutaraldehyde was addded dropwise with stirring. After 20 min at room temperature, the conjugate was purified on a Sephadex G-15 fine column.

The efficiency of the reaction was measured with ^{125}I coupled to the Tyr by the chloramine T method [24].

Glutaraldehyde cross-linked peptide was prepared in an analogous way [25].

Immunization of Animals

Immunization of rabbits was done as described [16] with 1 mg of peptide BSA conjugate suspended in 0.5 ml of 0.1 sodium phosphate buffer (pH 7.5) and 0.5 ml of complete Freund's adjuvant. The injection was repeated after 4 wk in the same manner.

After another 4 wk, a booster injection was given with glutaraldehyde cross-linked peptide. The animals were bled 2 wk after the last injection.

A rabbit hyperimmuneserum against purified SA11 (neutralized activity: 1:8,100) was kindly provided by Dr. H. Brüssow, Nestle Research Department, Vevey, Switzerland.

Enzyme-Linked Immunosorbent Assay (ELISA)

The reactivity of the antisera was determined by ELISA. Polystyrene plates were coated with 5–10 μg peptide or 50–100 ng of purified SA11 in 0.05 M CO_3^{2-} buffer (pH 9.6) per well.

Before adding the antibody dilutions, the plates were preincubated for 1 hr with a solution of 1% ovalbumin in sodium phosphate buffer. The unbound antibody was washed away, and a peroxidase conjugated secondary antibody was added for 30 min. Excess antibody was again washed away, and 0.04% o-phenylenediamine and 0.012% H_2O_2 in phosphate-citrate buffer pH 5.0 was added to the wells. The reaction was stopped with 1 M HCl. The extinction of the wells was measured in a Titertek Multiscan photometer at 492 nm.

Cells and Virus

Simian rotavirus SA11, which was a gift from Dr. P.A. Bachmann, University of Munich, was propagated in MA104 cells. For infection, the virus was activated with 10 μg trypsin/ml (Difco 1:250) at 37°C for 30 min. The cells were washed three times with Hank's Balanced Salt Solution (HBSS), and virus (20 $TCID_{50}$/cell) was adsorbed for 1 hr at 37°C. After washing with HBSS, the cells were maintained in Eagle's minimum essential medium (MEM) in the presence of 10 μg trypsin/ml in a roller apparatus at 37°C. Virus purification is described in detail elsewhere [26].

Preparation of Lysates from [^{35}S] Methionine-Labeled, SA 11-Infected Cells

Monolayers of MA104 cells in 95-mm plastic Petri dishes were infected with SA11. At 8 hr after infection, the cells were labeled for 4 hr with 50 μCi [^{35}S] methionine/ml (1,143.4 Ci/mmol, New England Nuclear) in methionine-free Eagles's MEM. The cells were then washed twice with ice-cold TD-buffer (25 mM Tris-HCl, pH 7.4, 5 mM KCl) and harvested in TD-buffer containing 0.02% EDTA. Cell lysates were prepared as previously described [27]. Lysates were centrifuged for 1 hr at 100,000g (Beckman; Airfuge).

Immunoprecipitation

Immunoprecipitation was done as previously described [27]. Briefly, 80 μl of cell extract was incubated with 5–20 μl of antiserum for 90 min at 4°C. One hundred microliters of 10% (v/v) Staphylococcus aureus suspension was added, and incubation was continued for an additional 30 min at 4°C. After washing, the complexes were cleaved by boiling the samples for 3 min in 50 μl of sample buffer.

Polyacrylamide Gel Electrophoresis

Fifteen percent polyacrylamide gels containing sodium dodecylsulfate were used for the separation of proteins as described previously [28,29].

The gels were dried, fluorographed [30], and exposed to presensitized Kodak X-Omat R films [31].

Purification of the Antipeptide Serum

The antipeptide serum was purified by affinity chromatography on 2 g of CH-Sepharose 4B to which 18 mg of peptide was coupled. Ten milliliters of serum was adsorbed to BSA-linked Sepharose, dialyzed against 20 mM Tris-HCl, pH 8.0, 29 mM NaCl, and applied to a DEAE-Affi-Gel Blue column (Bio-Rad).

The excluded IgG fraction was added slowly to the affinity column. The bound material could be eluted with 0.1 glycine solution (pH 2.5). The eluted fractions were immediately neutralized by the addition of Tris base.

RESULTS

Detection of Antipeptide Antibodies by ELISA

The development of antibodies was monitored by ELISA on platefixed octapeptide. A titer of 1:5,000 was reached by the antipeptide serum (data not shown). The preimmune serum did not bind to the peptide.

The reaction of the antipeptide serum with purified SA11 is shown in Figure 1. The antipeptide serum has a titer of at least 1:5,000.

The Antipeptide Serum Recognizes the VP7 Protein by Immunoprecipitation

The antipeptide serum was assayed for its ability to react with the VP7 protein by using [^{35}S] methionine-labeled extracts of SA11-infected MA104 cells. The immunoprecipitates were analyzed on NaDodSO$_4$/polyacrylamide gels. Preimmune-serum and serum from a nonimmunized rabbit were used as controls. As shown in Figure 2, lane d, the antipeptide serum precipitates the VP7, a glycoprotein with a molecular weight of 34,000 daltons. A polypeptide of apparent molecular weight of 42,000 daltons appearing nonspecifically in all immunoprecipitates represents the main inner capsid protein VP6.

Specificity of the Antipeptide Serum

The specificity of the antipeptide serum was demonstrated by competition experiments. Figure 3 shows that preincubation of the antipeptide serum with increasing amounts of peptide increased the inhibition of binding of the antipeptide serum to the polystyrene-bound SA11. In all experiments inhibition of more than 50% was achieved.

The immunoprecipitation of the VP7 can also be inhibited by addition of the octapeptide to the antipeptide serum (Fig. 4, lane d).

Cross-Reactivity of the Antipeptide Serum

Cross-reactivity of the antipeptide serum with human rotaviruses strain Wa (serotype 1) and Hochi (serotype 4) was determined by ELISA. The reaction of the antipeptide serum with both strains was positive up to a titer of 1:5,000. The reactivity

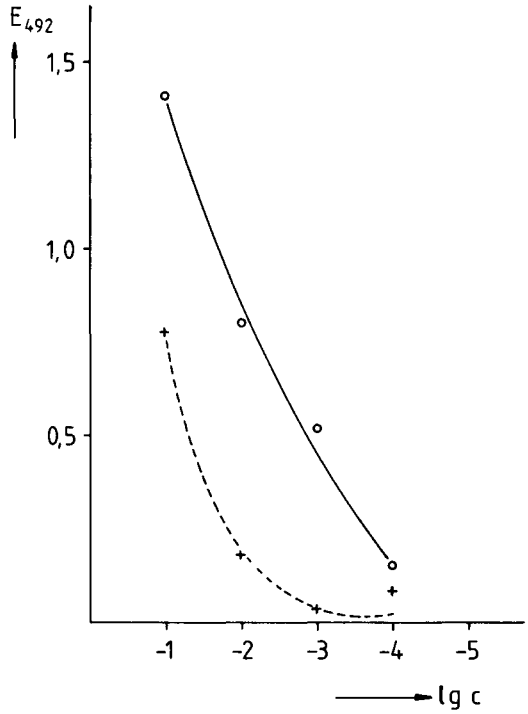

Fig. 1. Reaction of the antipeptide serum (○) and preimmune serum (+) with the SA11 virus in ELISA. Wells were coated with 100 ng/well of purified virus in 0.05 M CO_3^{2-} buffer pH 9.6.

of the antipeptide serum could be diminished by the addition of the octapeptide. Addition of a heptapeptide with a different sequence (Arg-Asn-Arg-Ser-Ser-Arg-Ser) did not show any effect.

DISCUSSION

The goal of the present investigation was to produce an antiserum against the outer capsid protein of SA11 by using a synthetic peptide corresponding to a specific region of the molecule. We chose a small region from the carboxy terminus of the VP7 protein, because it has the characteristics of a good antigen: it has polar residues, and it is located at the end of the polypeptide chain. Polar residues are usually located on the surface of native molecules, a prerequisite for interaction with antibodies. The carboxy and amino termini of a native protein may be conformationally less restricted than other parts of the molecule, therefore increasing the chance of being recognized by a larger fraction of the antibodies to the peptide [15,16].

The present study shows that the antipeptide serum recognizes the intact VP7 molecules in ELISA (Fig. 1) and by immunoprecipitation assay (Fig. 2), suggesting that the recognition is independent of the mode of presentation of the intact protein. The assays involve different extents of denaturation of the intact protein. In these assay conditions, however, the degree of chain folding is probably greater than the random folding of the peptide [32].

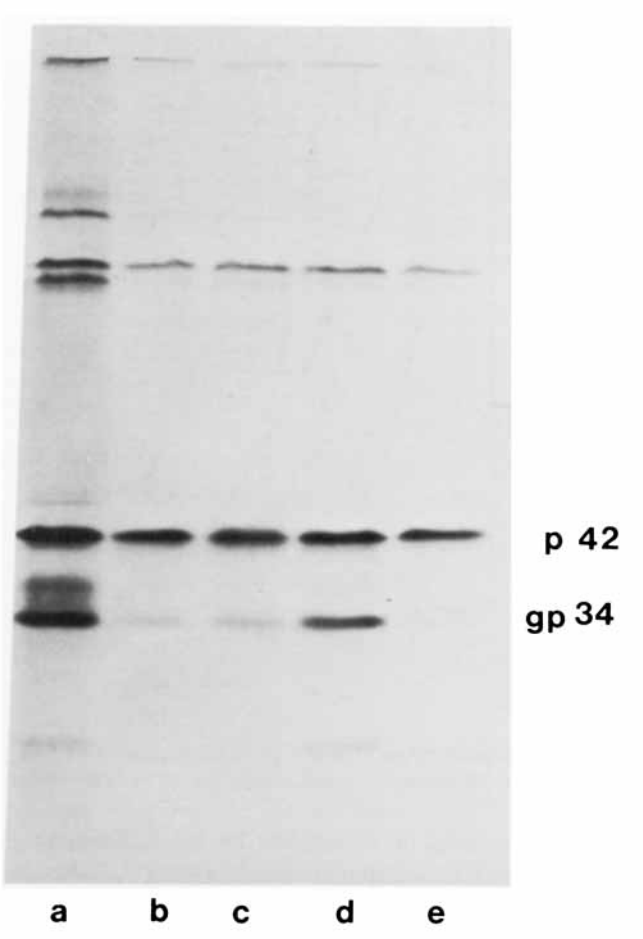

Fig. 2. Immunoprecipitation of the VP7 of SA11 (gp34) with antipeptide serum. [^{35}S] methionine-labeled extracts of SA11-infected MA104 cells were incubated with rabbit SA11 hyperimmuneserum (a), antipeptide serum after the first peptide injection (b), preimmune serum (c), antipeptide serum after booster injection (d), and serum from a nonimmunized rabbit (e). Antigen-antibody complexes were precipitated with Staphylococcus aureus and analyzed on 15% polyacrylamide gels.

The specificity of the antipeptide serum is confirmed by competition experiments between the octapeptide and the viral protein. Figure 3 shows that the binding of the antipeptide serum to the SA11 can be diminished by addition of the peptide. Figure 4 demonstrates that the immunoprecipitation of the VP7 can be inhibited by addition of the peptide to the antipeptide serum. As can be seen from Figures 2 and 4, the antipeptide serum, the preimmune serum, and serum from a nonimmunized rabbit react with the group-specific VP6. The same results were obtained when immunoprecipitations were performed according to the method of Lee et al. [33]. This agrees with the observations of other investigators [34] who have antibodies against SA11 VP6 in nearly every tested serum. The carboxy-terminal eight amino acids of the VP7 are highly conserved in different strains of rotaviruses. Therefore,

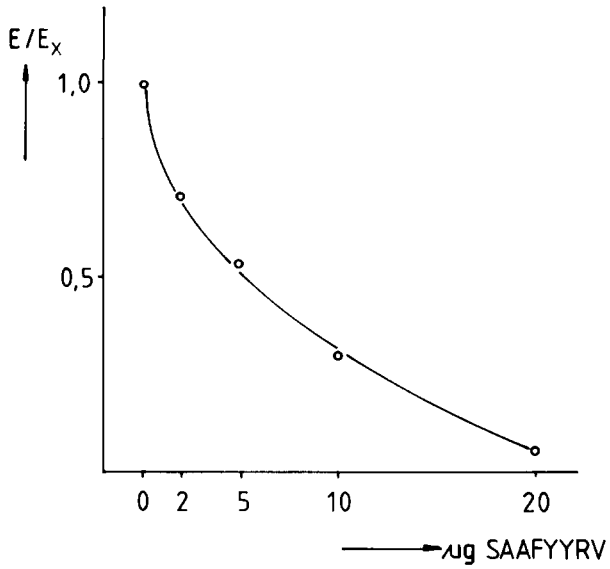

Fig. 3. Inhibition of the binding of antipeptide serum to plate-fixed SA11 (100 ng/well) by peptide. Preincubation of 1 ml antipeptide serum (dilution 1:100) with peptide was done overnight. E, absorbance at 492 nm with antipeptide serum preincubated with peptide; Ex, absorbance at 492 nm with antipeptide serum without addition of peptide.

the observed cross-reactivities of the antipeptide serum with the human strain Wa (serotype 1) and Hochi (serotype 4) were not surprising. Indeed, the identical synthesized sequence is found in the human serotype 1 [17,18]. The sequence of the VP7 from the human serotype 4 is not yet known. But owing to our results, there are probably no changes of essential amino acids [35] in the carboxy-terminus.

The results demonstrate that this part of the VP7 molecule may be a source of cross-reactivities between different strains if antibodies were directed against it. Indeed, when raising monoclonal antibodies against the SA11 and the Hochi strain, we found that 1 of 18 anti-SA11 monoclonal antibodies and 2 of 12 antibodies raised against the Hochi strain recognized the octapeptide (details of these experiments will be reported later [26]).

The antipeptide serum has no neutralizing activity (data not shown). The epitope(s) most critical for virus neutralization is (are) probably located on other parts of the molecule [17]. However, the antipeptide serum may be useful for localizing the VP7 in the infected cell and in virus particles. Moreover, it creates the ability of isolating VP7 molecules by affinity chromatography.

ACKNOWLEDGMENTS

The authors thank Anneliese Lapatz and Klaus Sure for excellent technical assistance.

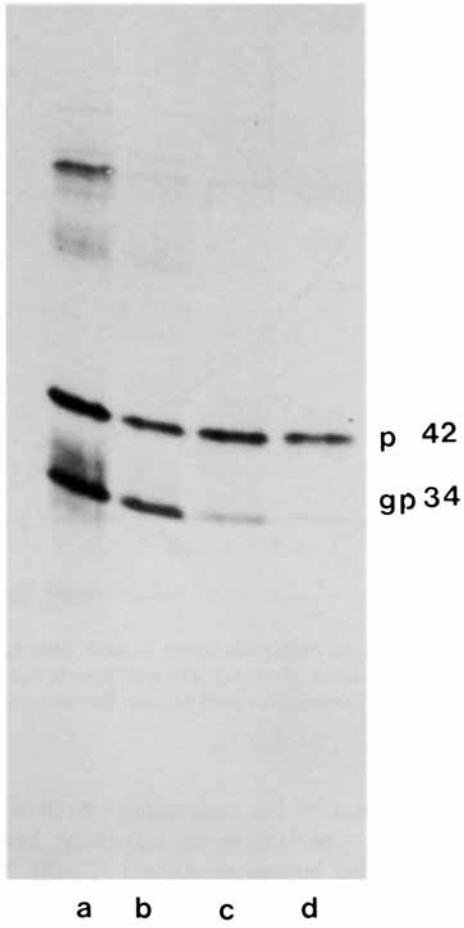

Fig. 4. Inhibition of the immunoprecipitation of the VP7 of SA11 with antipeptide serum. [^{35}S] methionine-labeled extract of SA11-infected MA 104 cells were incubated with rabbit SA11 hyperimmuneserum (a), affinity-purified antipeptide serum (b), antipeptide serum (c), and antipeptide serum preincubated with 10 μg of peptide (d).

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