Structure of rubella E1 glycoprotein epitopes established by multiple peptide synthesis

Brief Report

L. Lozzi¹, M. Rustici¹, M. Corti¹, M. G. Cusi², P. E. Valensin², L. Bracci¹, A. Santucci¹, P. Soldani¹, A. Spreafico¹, and P. Neri¹

¹ Dipartimento di Chimica and ² Istituto di Microbiologia, Università di Siena, Siena, Italy

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Summary. Essential parts of epitopes have been identified on rubella virion envelope glycoprotein E1, by scanning with overlapping octapeptides situated between amino acids 243–286 in a previously determined antigenic domain.

Rubella virus (RV), an enveloped positive-stranded RNA virus, is the only member of the genus Rubivirus within the family Togaviridae [7, 8]. The virus contains two glycosylated membrane-associated proteins, E1 and E2 and one non-glycosylated capsid protein, C [9, 12]. Sequence analysis indicates that the two different sizes of the E2 species are due to different glycosylated forms of the same polypeptide [6]. The E1 protein, which is 481 amino acids in length, has the characteristic features of an envelope glycoprotein, including potential N-linked glycosylation sites [1].

Previously it has been shown that both the haemagglutination inhibition and the neutralizing activity are associated with the E1 glycoprotein [4, 11]. Structure/activity relationships of this glycoprotein have been investigated by Ho-Terry et al. [5] and Terry et al. [10]: these authors, using specific monoclonal antibodies and cleavage peptides, were able to identify at least three epitopes within the 245–285 region of the E1 glycoprotein; in addition, antibodies recognizing two epitopes (EP₁ and EP₂) showed both haemagglutination inhibition and neutralizing activity, while antibodies recognizing the EP₃ epitope showed only neutralizing activity.

In order to establish the minimal linear sequences of these epitopes, we have synthesized the complete set of overlapping octapeptides, spanning the 243–
sequence of E1 RV glycoprotein (Fig. 1). Small amounts of peptides were simultaneously synthesized on activated polyethylene rods assembled in a 96-well microtiter plate pattern, using 9-fluorenylmethyloxycarbonyl (Fmoc)/t-butyli protecting group combination (Cambridge Research Biochemicals, Cambridge, England) [2, 3]. Removal of the Fmoc group was obtained by 20% piperidine in N,N-dimethylformamide and the amino acid carboxyl group was activated as pentafluorophenyl ester in 1-hydroxybenzotriazole solution. Once synthesis had been completed, the N-terminus of each peptide was acetylated to remove unnatural charges and finally amino acid side chains were deprotected using a mixture of trifluoroacetic acid/phenol/ethanedithiol (95/2.5/2.5).

Synthesis was monitored by amino acid analysis of control peptides concurrently prepared on adjacent rods.

Peptide/antibody interactions were detected using peptides linked to rods directly in an immunoenzymatic assay.

Briefly, peptide-coated rods were further coated with 1% ovalbumin/1% bovine serum albumin/0.1% Tween 20 in phosphate-buffered saline pH 7.2 (PBS) for 1 h at 25 °C, incubated overnight at 4 °C in a suitable dilution (32 µg/µl) of pooled human high titre anti-rubella IgG from subjects hyperimmunized