Chromatographic Studies on Picornavirus Capsid Polypeptides

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SUMMARY

The polypeptides of encephalomyocarditis, Mouse-Elberfeld and type 5 rhinoviruses behave similarly when chromatographed on calcium phosphate (brushite), each being eluted by a linear phosphate buffer gradient containing sodium dodecyl sulphate in three major peaks, C1, C2 and C3. Analysis of the peaks by polyacrylamide gel electrophoresis suggests that the major capsid polypeptides of these three picornaviruses elute in the order: δ (peak C1), γ with β (peak C2) and α (peak C3).

The capsid of a large plaque variant of the picornavirus, encephalomyocarditis (EMC) virus comprises four major polypeptides, α, β, γ, and δ of mol. wt. 32,000, 32,000, 25,500 and 6,700 respectively (Burness, Fox & Pardoe, 1974). Chromatography of these polypeptides on calcium phosphate (brushite) in the presence of sodium dodecyl sulphate (SDS) resulted in elution of γ at the same phosphate molarity as a 32,000 mol. wt. polypeptide (Burness et al. 1974), but whether the latter was α or β was unknown since no method was available for distinguishing between the two. To resolve this problem, we have examined the chromatographic properties of polypeptides from a rhinovirus which, like EMC, is a picornavirus but belongs to a different subgroup (International Enterovirus Study Group, 1963), the α and β polypeptides of which are separable by SDS-polyacrylamide gel electrophoresis (Medappa, McLean & Rueckert, 1971; Stott & Killington, 1973). We have also compared the chromatographic properties of polypeptides from Mouse-Elberfeld (ME) virus with those of EMC virus; both of these viruses belong to the same subgroup.

A large plaque variant of EMC virus and the M2 variant of ME virus (seed kindly supplied by Dr R. R. Rueckert) were both grown in Krebs II ascites tumour cells (Sanders, Huppert & Hoskins, 1958) in the presence of 2 μCi/ml [3H]-labelled amino acids (New England Nuclear, Boston, Mass., 02118) and purified as described previously (Burness, 1969a). The Norman strain of rhinovirus type 5 was grown in HeLa cells in the presence of 2.0 μCi/ml [14C]-protein hydrolysate (The Radiochemical Centre, Amersham, England) and purified by sucrose density gradient sedimentation (Stott & Killington, 1973).

Radioactivity in all three purified viruses, disrupted by boiling in 1% (w/v) SDS containing 4 m-urea and 1 mm-dithiothreitol (DTT), was eluted from calcium phosphate columns prepared and used as previously described (Burness et al. 1974), in three major peaks termed C1, C2 and C3 (Fig. 1); a small, variable amount of material was also frequently detected in the void volume. In some experiments, peak C1 showed some heterogeneity, particularly that from rhinovirus. Peak C2 from all three viruses invariably appeared heterogeneous; attempts to resolve the components in EMC virus peak C2 by chromatography using more complex gradients were unsuccessful although two components are separable by SDS-gel electrophoresis (A. T. H. Burness & I. U. Pardoe, unpublished data). Peak C3 from all three viruses eluted as a sharp symmetrical peak and thus appeared to be homogeneous.

The mean and standard error of the mean of the phosphate concentrations for elution of EMC virus peaks C1, C2 and C3, based on 7 chromatographic runs, were 0.053 M ± 0.002, 0.153 M ± 0.005 and 0.22 M ± 0.003, respectively. These values were similar to those required for elution of the equivalent ME virus peaks for which the mean of five runs gave 0.047 M ±
Short communications

Fig. 1. Calcium phosphate chromatographic profiles of disrupted [³H]-amino acid labelled (a) EMC and (b) ME viruses and (c) [¹⁴C]-amino acid labelled rhinovirus type 5. These purified viruses were disrupted into their constituent polypeptides by boiling for 10 min in 1 % (w/v) SDS containing 4 M-urea and 1 mM-DTT. The calcium phosphate in a glass column 2.5 cm in diam. and 20 cm long was subjected to elution with a linear gradient prepared by mixing 100 ml each of 0.005 M- and 0.3 M-sodium phosphate buffer, pH 7.0 containing 1 % (w/v) SDS and 1 mM-DTT throughout. Flow rates were 1 ml/min or less for optimum resolution and 2 ml fractions collected of which 1.5 ml was added to 10 ml cocktail D (380 g naphthalene and 19 g diphenyloxazole made up to 3.71 with dioxane) for scintillation counting (-----); phosphate concentrations (------) were measured by a modified method of Fiske & Subbarow (1925), as previously described (Burness, 1969b).

0.003, 0.151 M ± 0.013 and 0.221 M ± 0.005 phosphate. The concentrations for elution of the equivalent type 5 rhinovirus peaks, based on two determinations only, were lower (0.02 M) for C1, about the same (0.15 M) for C2 and higher (0.245 M) for C3.

The amount of radioactivity eluted in peaks C1, C2 and C3 for ME and rhinoviruses was generally similar to that in equivalent EMC virus peaks particularly considering the difficulties in deciding on the exact location of the base line. The mean and standard error of the mean (3 determinations for each virus) of the percentage of radioactivity in peaks C1, C2 and C3 was 8.7 ± 0.7, 60.7 ± 0.7, and 30.5 ± 0.5 respectively, for EMC virus, 10.0 ± 0.4, 59.2 ± 4.2 and 28.5 ± 4.0 for ME virus and 7.4 ± 0.2, 58.5 ± 2.1 and 34.1 ± 2.2 for type 5 rhinovirus.
Fig. 2. SDS-gel electrophoresis of [3H]-amino acid labelled (a) EMC and (b) ME viruses and (c) [14C]-amino acid labelled rhinovirus. The viruses were disrupted as detailed in Fig. 1 and electrophoresed in 10% (w/v) polyacrylamide gel containing 0.1% (w/v) SDS, as described by Dunker & Rueckert (1969). The position of the radioactive polypeptides was estimated by pulverizing the gels in a Gilson gel fractionator (Gilson Medical Electronics, Middleton, Wisconsin 53562, U.S.A.) and diluting the fractions so obtained with 1 ml 0.1% (w/v) SDS before addition of 10 ml cocktail T (5 g diphenylxazole in 200 ml Bio-Solv BBS-3, from Beckman Instruments Inc., Fullerton, California 92634, U.S.A., and 11 toluene) for scintillation counting.

The radioactivity in SDS-disrupted ME or rhinoviruses was separated into four major peaks, α, β, γ, δ by SDS gel electrophoresis, whereas EMC virus polypeptides α and β were not resolved, as has been shown previously (Burness et al. 1974) and, therefore, three major peaks only were apparent for this virus (Fig. 2). This single result for rhinovirus type 5 differed in two respects from a previous study (Stott & Killington, 1973) in that, unlike before, polypeptides β and γ were resolved here but at the same time there appeared to be some loss of polypeptide α.

Using EMC virus polypeptides, the sizes of which we had previously determined (Burness et al. 1974), as standards electrophoresed in parallel tubes, mol. wt. for ME and rhinovirus...
polypeptides were estimated to be: $\alpha = 33000$ and $33060$, respectively, (compared with 32000 for EMC), $\beta = 30800$ and 30450 (EMC = 32000), $\gamma = 25300$ and 26900 (EMC = 25500) and $\delta = 10900$ and 8550 (EMC = 11000 by electrophoresis). As discussed previously (Dunker & Rueckert, 1969; Burness et al. 1974), mol. wt. for proteins of about 10000 or less may be in error when estimated by electrophoresis on 10% gels. Therefore, these values for the mol. wt. of the $\delta$ polypeptide for all three viruses should be treated with caution.

The polypeptide composition of the chromatography peaks were analysed by SDS-gel electrophoresis. It has been shown previously that EMC virus peak C1 is composed solely of polypeptide $\delta$ (Burness et al. 1974) and the same is assumed for ME and rhinoviruses based on similarities with the EMC chromatographic profile (Fig. 1) and the percent radioactivity present in this peak for all three viruses (see above). To determine which polypeptides were present in peaks C2 and C3, samples comprising each peak obtained by chromatography of disrupted [14C]-labelled rhinovirus were concentrated and desalted by dialysis against 0.1% (w/v) SDS in a micro-ultrafiltration system (Model 8 MC, Amicon Corp., Lexington, Mass., U.S.A.) fitted with a PM 10 membrane. Subsequent SDS-gel electrophoretic analysis of peak C3 revealed the presence mainly of a single component of mol. wt. about 33000 which was assumed to be polypeptide $\alpha$, whereas peak C2 was found to contain two incompletely separated components with mol. wt. of about 27000 and 30000, thus corresponding to polypeptides $\gamma$ and $\beta$ (Fig. 3). Since the chromatographic profiles of EMC and rhinoviruses were similar (Fig. 1), we assume that the same situation prevails for EMC virus and that peak C2 comprises polypeptides $\beta$ and $\gamma$ while polypeptide $\alpha$ constitutes peak C3.

The molar ratios of polypeptides $\alpha: \beta+\gamma: \delta$ have been reported to be about $1:2:0.5$ for rhinovirus type 5 (Stott & Killington, 1973) and $1:2:1$ for rhinoviruses A1, 2 and 4 (Medappa...
et al. 1971). From the proportion of radioactivity in the chromatographic peaks and the mol. wt. of the polypeptides (see above), the molar ratios of \( x : \beta + \gamma : \delta \) were calculated to be about \( 0.9 : 2.0 : 0.8 \) for EMC virus, \( 0.8 : 2.0 : 0.9 \) for ME virus and \( 1 : 2.0 : 0.9 \) for type 5 rhinovirus.

Separation of polypeptides by chromatography on calcium phosphate (brushite) in the presence of SDS appears generally related, with some anomalies, to the size of the polypeptides (A. T. H. Burness & I. U. Pardoe, unpublished data). Whatever property results in anomalous behaviour appears common to the \( \beta \) polypeptides from all three picornaviruses examined in this study: in each case, \( \beta \) of mol. wt. about 30000 to 32000 was apparently eluted with polypeptide \( \gamma \) of mol. wt. about 25000 to 27000. It is possible that the \( \beta \) polypeptides from all three viruses possess similar amino acid sequences in part which directly or indirectly, for instance through their effect on secondary structure or SDS binding, result in anomalous chromatographic properties using the system described here. If this possibility is correct, it suggests that the particular sequences involved must be of critical importance to have been conserved in the \( \beta \) polypeptides of picornaviruses of different subgroups.

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Memorial Sloan-Kettering Cancer Center
New York, New York 10021, U.S.A.
Clinical Research Centre
Harrow, Middlesex HA1 3UJ, England

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