BIOCHEMICAL EVIDENCE FOR INTERTYPIC GENETIC RECOMBINATION OF POLIOVIRUSES

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1. Introduction

Two basically different kinds of genetic recombination are known to occur in RNA-containing viruses. Firstly, there is a high-frequency recombination among viruses with segmented RNA genomes, such as reoviruses [1] or orthomyxoviruses [2]. This type of genetic recombination is due to physical exchange of separate RNA molecules ('segments') between parental genomes and, therefore, may be designated 'genetic reassortment' [3]. Secondly, relatively low-frequency recombination is reported to exist in picornaviruses, whose genome is represented by a single RNA molecule [4,5]. The nature of the latter type of recombination is poorly understood, but it is believed to be true inheritance in a single RNA molecule of genetic properties of both parents [4,5]. Evidence for this interpretation has only been provided by purely genetic techniques and direct biochemical proof that recombinants do acquire genetic information from more than one parent is lacking. Experimental solution of this problem became more feasible when recombinants between different poliovirus serotypes were obtained (E. A. T., M. S. K., in preparation). Well-established differences between the genomes of different types of polioviruses ([6–10], L. I. R., E. A. T., V. I. A., submitted) make possible a direct comparison of recombinants with each of their parents.

Here we report results showing that some polypeptides coded for by the genome of a type 1/type 3 poliovirus recombinant are indistinguishable from the corresponding polypeptides of the type 1 parent, whereas some other recombinant-specific polypeptides appear to be of the type 3 origin. These results are the first direct biochemical evidence for intermolecular recombination between RNA genomes.

2. Methods

Poliovirus type 1, Mg^rS7^r, is a two-step derivative of strain Mahoney, which was selected consecutively for resistance to guanidine–HCl (60 μg/ml) and ethyl-2-methylthio-4-methyl-5-pyrimidine carboxylate (S7, 100 μg/ml). It was cloned twice in the presence of S7. Poliovirus type 3, *ts*557, is a cloned two-step derivative of strain 452/62IIID (originally isolated from a human polio case); first, a *tr* mutant of the latter strain was selected by growing the virus at 40°C, and then a *ts* mutation was introduced by 5-fluorouracyl mutagenesis. Genetic experiments (to be reported elsewhere) allowed us to place the *ts*557 mutation between the guanidine locus and the 3′-end of the genome (fig.1).

The recombinant, Mg^rS7^r/*ts*557, was obtained upon mixed infection of HeLa cells with parental viruses at a multiplicity of infection with each virus of ~20 p.f.u./cell. Intertypic recombinants in the progeny of the mixed infection were scored by plaque assay in *Macaca rhesus* kidney cell cultures under conditions restrictive for either parent alone, namely, in the presence of an antiserum against polio type 1 (inhibiting the type 1parent) and guanidine–HCl (60 μg/ml); in addition, the assay was carried out at 40°C (both latter conditions were inhibitory for the type 3 parent).

Labeling of virus-specific polypeptides was achieved by incubation of virus-infected HeLa cells with [^{35}S]methionine from 2–4 h postinfection. The cytoplasmic extracts were prepared by treating the cell suspension in 0.15 M NaCl, 5 mM EDTA, 50 mM Tris–HCl (pH 7.8), 0.02% Na-azide with 1% NP-40 at 4°C for 5 min followed by low-speed centrifugation. As a source of capsid polypeptides, preparations

of virions purified essentially as in [11] were used. Partial proteolysis products were analysed essentially as in [12,13]. The following proteolytic enzymes were utilized: a protease from *Streptomyces caespitosus*, chymotrypsin, and papain.

3. Results and discussion

Some phenotypic properties of the type 1/type 3 polio recombinant used in this study are presented in table 1. It is clearly seen that the neutralization properties as well as sensitivity to S7, a non-selected capsid polypeptide marker [4], both indicate that at least some capsid polypeptides of the recombinant are inherited from the type 3 parent, whereas the resis-

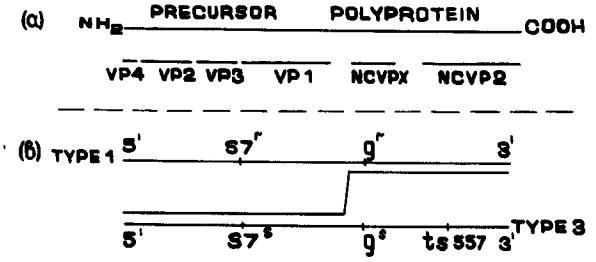

Fig.1. (a) Approximate positions of the sequences corresponding to some capsid and non-capsid polypeptides in the poliovirus polyprotein. (b) Schematic representation of the parental and recombinant genomes. Positions of the genetic loci are given arbitrarily, but the S7 and *ts*557 loci are known to be situated in the capsid and non-capsid regions of the genome, respectively, and the *g*-locus should be placed between the S7 and *ts*557 sites.

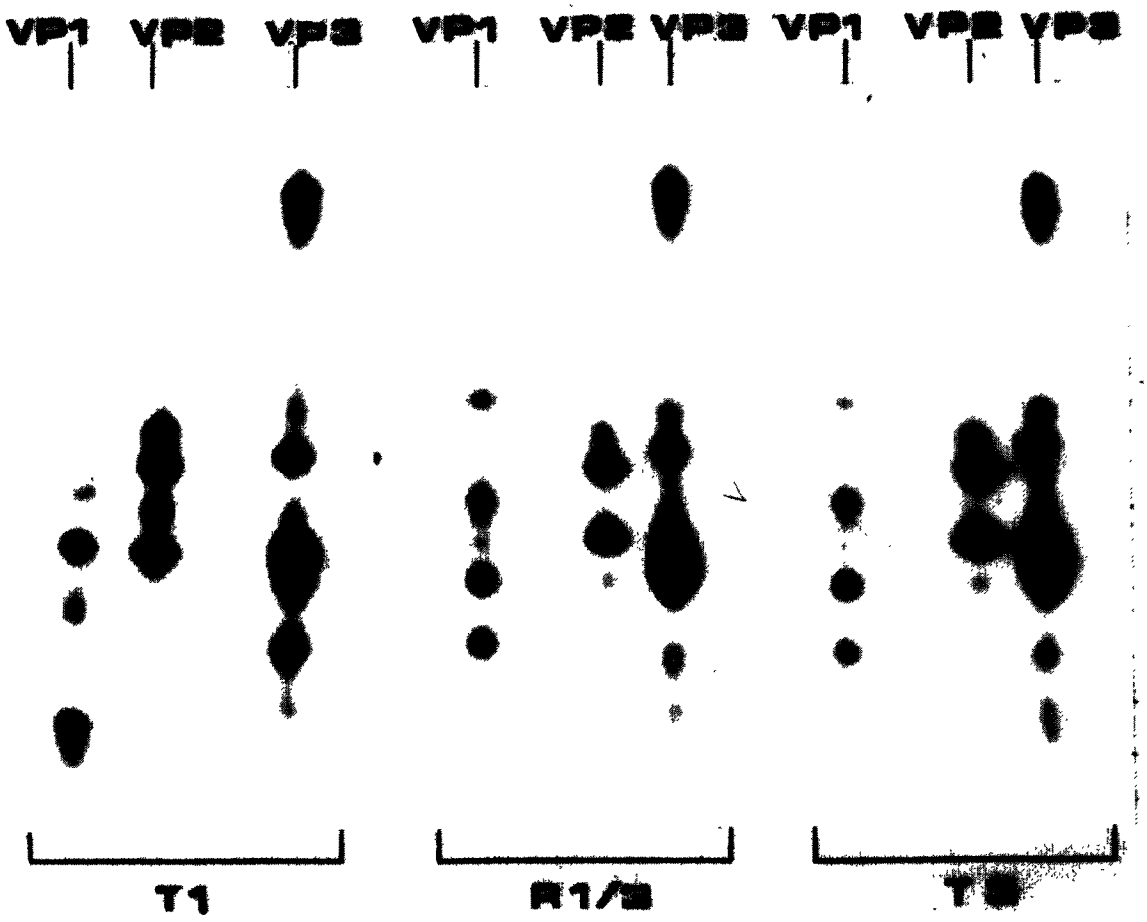

Fig.2. Partial proteolysis products of the capsid polypeptides. Purified preparations of virions of poliovirus type 1 (T1), poliovirus type 3 (T3), and recombinant $Mg^rS7^r/ts557$ (R1/3) were subjected to electrophoresis in a SDS-containing polyacrylamide slab, gel regions containing capsid polypeptides were excised, treated with a protease from *S. caespitosus*, and the resulting products were subjected to the second-dimension electrophoresis.

tance to guanidine and the *tr* phenotype are similar to those of the type 1 parent. A schematic representation of the recombinant genome which is compatible with the data of table 1, is given in fig.1.

One can argue, however, that the phenotypic properties of Mg^{r}S7^r/*ts*557 may, in principle, be explained by assuming that the type 3 parent had not undergone, upon mixed infection, any recombinational event, but rather two, or even one, mutations rendered the virus both guanidine- and temperature-resistant. Against the latter interpretation two genetic observations may be adduced:

(i) The number of plaques formed under conditions restrictive for either parent in the progeny of the type 1/type 3 mixed infection was ~15-fold higher (~9×10^{-5}) than in the progeny of the single type 3 infection (~6×10^{-6});

(ii) The clones of type 3 virus which were selected for the ability to reproduce in the presence of guanidine were so far invariably found to be guanidine-dependent (i.e., requiring guanidine for reproduction) rather than guanidine-resistant (i.e., capable of reproducing both in the presence and the absence of guanidine).

On the other hand, Mg^{r}S7^r/*ts*557 displayed a guanidine-resistant phenotype (table 1) and it is only natural to assume that it acquired this phenotype from its type 1 parent. Although this argument may suggest that the virus with the combined properties of the type 1 and type 3 parents is actually a recombinant, direct biochemical proof of this notion is desirable.

If the genome of our provisional recombinant is properly outlined in fig.1, then it should have capsid polypeptides of the type 3 parent and at least some non-capsid polypeptides of the type 1 virus. Partial proteolysis mapping allowed us to distinguish capsid as well as some non-capsid polypeptides of polioviruses belonging to different serotypes (L. I. R., E. A. T., U. I. A., submitted). Therefore, using this technique we have compared capsid (VP1, VP2 and VP3) and non-capsid (NCVP2) polypeptides of the three viruses, Mg^{r}S7^r/*ts*557 and both its presumptive parents.

The results obtained with the protease from *S. caespitosus* are presented in fig.2,3. The capsid polypeptides of the recombinant are very, similar, perhaps identical, to those of the type 3 virus (fig.2). On the other hand, the NCVP2 polypeptide of the recombinant is indistinguishable from the corresponding polypeptide of its type 1 parent, but reveals repro-

Table 1
Phenotypic properties of the recombinant and its parents[a]

Conditions of assay	Type 1	Type 3	Recombinant
Control	⩾376	74	⩾56
Antipolio 1 serum	<4	⩾80	⩾60
Antipolio 3 serum	⩾400	<1	<0.5
S7	⩾400	<1	<0.5
Guanidine	⩾400	<1	⩾57
40°C	⩾400	<1	⩾53

[a] Stocks of the three viruses were plaque-titrated in *M. rhesus* kidney cell cultures at 37°C, except the last line. The results are expressed in p.f.u./ml × 10^{-6}. ⩾ indicates that a relatively high number of plaques developed in each flask, so that some plaque overlapping could not be excluded. Concentrations of the inhibitors are listed in section 2

Fig.3. Partial proteolysis products of the NCVP2 polypeptides. Labeled extracts from the cells infected with the appropriate virus were subjected to electrophoresis in a SDS-containing polyacrylamide slab, gel regions containing NCVP2 were excised, treated with a protease from *S. caespitosus*, and the resulting products were subjected to electrophoresis.

ducible differences with NCVP2 of the type 3 virus. Similar results were obtained with the three proteases utilised. This is direct evidence for intermolecular genetic recombination among RNA genomes.

Another point also deserves comment. It is usually assumed that the locus responsible for the sensitivity of poliovirus reproduction to guanidine is located in the capsid region of the genome [4]. However, the recombinant studied here appears to acquire the guanidine locus from one parent and capsid polypeptides including VP1 (the most 5′-end distal capsid polypeptide) from the other. This finding suggests that the guanidine locus lies outside the capsid region [5], but more definitive statement to this end requires detailed studies of different polypeptides. Such studies aimed at more precise localisation of the guanidine locus and some other genetic loci, using this approach, are in progress.

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