Gene Order of the Poliovirus Capsid Proteins

DAVID REKOSH

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received for publication 2 November 1971

Two methods were used to determine the genetic map of the poliovirus capsid proteins. The first method uses pactamycin, a drug which selectively inhibits the initiation of protein synthesis and causes a change in the relative amounts of capsid proteins synthesized. This differential effect on each of the capsid proteins is interpreted as indicating the relative distance of each protein from the initiation site of protein synthesis. The second method involves an analysis of coat precursor molecules released from polyribosomes after a series of short pulses of different length terminated by addition of emetine, a drug which stops all protein synthesis almost immediately after its addition. As the pulse length is increased, each of the capsid proteins within the precursor gains radioactivity with different kinetics. From these kinetics, it is possible to determine the gene order of the capsid proteins within the precursor as well as a rate of protein synthesis. Both methods indicate a gene order for the region of the ribonucleic acid coding for the capsid proteins as (5' → 3') VP 4 — VP 2 — VP 3 — VP 1.

Poliovirus contains a single strand of ribonucleic acid (RNA) which directs the synthesis of a number of proteins. A technique to determine the position of the regions of viral RNA encoding some of these proteins, using the drug pactamycin, was recently described (16). This technique is based on several observations. (i) It appears that there is only a single site for the initiation of protein synthesis on the poliovirus genome and that the various protein species found in the infected cell arise by proteolytic cleavage from one large precursor polypeptide (2, 10, 12; D. Baltimore et al., *The Strategy of the Viral Genome, in press*). (ii) Pactamycin, at a proper concentration, is a selective inhibitor of the initiation of protein synthesis in poliovirus-infected HeLa cells (16). (iii) Synthesis of protein initiates near the 5' end of the RNA and proceeds towards the 3' end (17).

Given these facts, inhibition of the initiation of protein synthesis by pactamycin should be followed by a transient period of protein synthesis during which the relative amounts of the species of proteins synthesized reflect their position on the viral genome. The protein encoded by the 5' terminal end of the RNA should be the most reduced, followed in order by the proteins encoded by regions increasingly nearer the 3' end. In this way, the sequence of the three major cleavage products was provisionally determined (16). Confirmatory results have been reported (D. F. Summers and J. V. Maizel, Jr., *Proc. Nat. Acad. Sci. U.S.A., in press*).

NCVP 1, which is the precursor of the four viral

coat proteins (VP 1, VP 2, VP 3, VP 4; reference 13), was found to occur at or near the 5' end of the poliovirus genome. In this paper, I describe two types of experiments which allow the order of the four viral coat proteins which constitute NCVP 1 to be determined. The first type of experiment is a direct extension of the pactamycin technique described above. The second method, which utilizes the drug emetine, is described below.

Emetine is an extremely potent inhibitor of protein synthesis in HeLa cells. After administration of the drug, the nascent polypeptide chains remain attached to the polyribosomes, and amino acid incorporation is inhibited by greater than 99% (6, 7, 15). A short pulse of radioactivity, followed by the addition of emetine, will therefore result in the release from the polyribosomes of only a few completed radioactive NCVP 1 molecules. Since uncompleted molecules remain attached to the polyribosomes, the completed ones will be the only molecules available to be cleaved into the virion proteins. If the length of the pulse is short, there should be a differential labeling of the coat proteins within NCVP 1. Since the viral RNA is translated in a direction of 5' to 3', a very short pulse of radioactivity should label only that coat protein which maps at the 3' end of the region of the RNA coding for NCVP 1. Increasingly longer pulses should label coat proteins which map increasingly closer to the 5' end of this region. Thus, by analyzing completed virions made from protein synthesized during pulses of various length, it

should be possible to determine a gene order for the viral coat proteins.

The results of both types of experiments presented here are consistent and allow the unambiguous ordering of the genetic map for the poliovirus coat proteins as (5' → 3') VP 4 — VP 2 — VP 3 — VP 1. Furthermore, from the kinetics of appearance of radioactivity in VP 2 and VP 3, a rate of protein synthesis of approximately 200 amino acids per min can be calculated.

MATERIALS AND METHODS

Infection of cells and purification of virus. The growth of poliovirus and its purification by centrifugation through a sucrose gradient have been previously described (3).

Gel electrophoresis. Purified virus was disrupted by boiling for 5 min in a solution of 1% mercaptoethanol, 1% sodium dodecyl sulfate (SDS), and 15% glycerol. It was then subjected to the Maizel and Laemmli modification of SDS-polyacrylamide gel electrophoresis (14: J. V. Maizel, *personal communication*) for 3.5 hr at 100 v. The 10% separating gel contained a ratio of acrylamide to *N,N'*-bis methylene acrylamide of 18.7:1.

Materials. ^3H -leucine (39 Ci/mmol) and ^{14}C -leucine (260 mCi/mmol) were obtained from New England Nuclear Corp. Pactamycin was a gift of Gunther Fonken of the Upjohn Co. Emetine was obtained from the Sigma Chemical Co.

RESULTS

Effect of pactamycin on the viral coat proteins.

A slightly higher concentration of pactamycin was used in these studies than previously described (16) because this concentration was found to inhibit the initiation of protein synthesis more completely (D. Rekosh, *unpublished data*). The concentration used, however, is still two-fold less than that described by others for the inhibition of initiation in poliovirus-infected HeLa cells (Summers and Maizel, *Proc. Acad. Nat. Acad. Sci. U.S.A.*, *in press*).

To investigate the effect of pactamycin on the synthesis of the four viral coat proteins, HeLa cells infected for 3 hr with poliovirus were exposed to 5×10^{-7} M pactamycin and, after 1 min 15 sec, ^3H -leucine was added. After 30 min, a 1,000-fold excess of unlabeled leucine was added to minimize labeling of the virion due to "leakage" caused by a possible incomplete inhibition of initiation. At 6 hr after infection, the cells were harvested, a cytoplasmic extract was prepared and treated with SDS and virions were purified by rate zonal centrifugation in a sucrose gradient. To produce control virus, a parallel infection which did not receive pactamycin was labeled for 3 hr with ^{14}C -leucine and treated similarly.

Figure 1a shows a profile of a typical sucrose gradient from a purification of virions labeled in the presence of pactamycin. The ability to produce

labeled virus under these conditions demonstrates that an inhibition of protein synthesis does not interfere with the maturation of previously synthesized protein into virions, a result previously observed when using cycloheximide (3). Peak fractions were pooled and the virions were recovered by centrifugation.

To analyze the proteins of the virions labeled with ^3H -leucine in the presence of pactamycin, the virions were combined with similarly purified ^{14}C -leucine-labeled control virions, disrupted, and submitted to electrophoresis on a polyacrylamide gel (Fig. 1c). The data shown were normalized so that the peaks of VP 1 were the same height. Relative to the radioactivity in VP 1, pactamycin slightly reduced the radioactivity in VP 3, more severely reduced VP 2, and abolished the labeling of VP 4.

To investigate the possibility that the observed differential labeling of the capsid proteins was due to induction by pactamycin of specific degradation of certain proteins or of differences in the behavior of protein pools, the following experiment was performed. At 3 hr after infection, cells were exposed to ^3H -leucine for 7 min. A 1,000-fold excess of nonradioactive leucine was then added along with pactamycin to a concentration of 5×10^{-7} M, and the infection was allowed to continue until 6 hr, at which time the cells were harvested and labeled virions were purified. Virions obtained from this experiment were mixed with ^{14}C -labeled control virions, disrupted, and submitted to electrophoresis (Fig. 1b). When the VP 1 peaks were normalized to the same height, the peaks of VP 2, VP 3 and VP 4 coincided, indicating that pactamycin had had no effect on the relative amounts of prelabeled capsid proteins appearing in complete virions.

To confirm that VP 4 was most severely affected by pactamycin, the time between the administration of the drug and the addition of labeled leucine was shortened. At 3 hr after infection, pactamycin was added to 5×10^{-7} M, and 10 sec later a 30-sec pulse of ^3H -leucine was given. Virions were purified after 6 hr of infection, and their protein was analyzed by electrophoresis, along with a control preparation labeled with ^{14}C -leucine (Fig. 1d). The only noticeable difference between these two samples is an approximately 50% reduction of the VP 4 peak in the preparation treated with pactamycin.

Analysis of capsid proteins released from polyribosomes during short pulses. Interpretation of the data from the pactamycin experiments described above allows one to determine the gene sequence of the poliovirus coat proteins (*see below*). The experiments reported in this section provide another method for arriving at a gene sequence.

HeLa cells infected for 3 hr with poliovirus were

FIG. 1. Effect of pactamycin on poliovirus capsid proteins. (a) Sucrose gradient profile of virions synthesized in the presence of pactamycin. At 3 hr after infection, cells were resuspended in Earle's saline plus 5% dialyzed horse serum. Pactamycin was added to 5×10^{-7} M, and 10 sec later ^3H -leucine at $10 \mu\text{Ci/ml}$ was added. Thirty minutes after the addition of pactamycin, a 1,000-fold excess of nonradioactive leucine was added. At 6.5 hr after infection, cells were harvested, a cytoplasmic extract was made, and virus was purified by centrifugation through a linear 15 to 30% sucrose gradient containing 0.5% sodium dodecyl sulfate, 0.01 M tris(hydroxymethyl)aminomethane, 0.001 M ethylenediaminetetraacetic acid, 0.01 M NaCl. The gradient was centrifuged for 2.75 hr at 27,000 rev/min in a Spinco SW27 rotor. Fractions were taken, and portions of them were counted in a Beckman scintillation counter. (b) Polyacrylamide gel analysis of virion proteins labeled before pactamycin addition. Cells were treated similarly to (a), except that after resuspension ^3H -leucine to $10 \mu\text{Ci/ml}$ was added 7 min before the addition of both pactamycin and a 1,000-fold excess of nonradioactive leucine. To prepare control virions, $0.3 \mu\text{Ci}$ of ^{14}C -leucine per ml was added to resuspended cells at 3 hr after infection in the absence of pactamycin, and virions were purified after 6 hr of infection. The fractions from a sucrose gradient similar to (a) were pooled as indicated, pelleted, and subjected to electrophoresis as described in the text. Radioactivity was normalized to 100 in fraction 25. This represents 2,019 counts/min of ^3H and 4,223 counts/min of ^{14}C . Symbols: Δ , ^3H -labeled experimental sample; $\bullet$, ^{14}C -labeled control. (c) Polyacrylamide gel analysis of virion proteins labeled after pactamycin addition. Cells were treated similarly to (a) and (b) except that ^3H -leucine was added to $10 \mu\text{Ci/ml}$ at 1 min 15 sec after the addition of pactamycin. After 28 min 45 sec, a 1,000-fold excess of nonradioactive leucine was added. Radioactivity was normalized to 100 in fraction 39, representing 827 counts/min of ^3H and 1,440 counts/min of ^{14}C . Symbols: Δ , ^3H -labeled experimental sample; $\bullet$, ^{14}C -labeled control. (d) Polyacrylamide gel analysis of virion proteins labeled just after pactamycin addition. Cells were treated the same as in (a) and (b), except that ^3H -leucine was added to $10 \mu\text{Ci/ml}$ at 10 sec after the addition of pactamycin. Forty seconds after the addition of pactamycin, a 1,000-fold excess of nonradioactive leucine was added. Radioactivity was normalized to 100 in fraction 38, representing 887 counts/min of ^3H and 855 counts/min of ^{14}C . Symbols: Δ , ^3H -labeled experimental sample; $\bullet$, ^{14}C -labeled control.

exposed to pulses of ^3H -leucine for 2, 3, 4, 5, 6, 10, or 15 min. The pulses were terminated by the addition of emetine at a final concentration of $100 \mu\text{g/ml}$. This concentration has been shown to inhibit total amino acid incorporation into HeLa cells by more than 99% within 10 sec (7, 15; unpublished data). The infection was then allowed to continue until 6 hr when labeled virions were purified, mixed with ^{14}C -labeled control virions,

disrupted, and analyzed by electrophoresis. The data were normalized to the height of the VP 1 peak.

Figure 2a shows the data from the 2-min pulse. Most of the radioactivity was found in the VP 1 peak. The VP 3 peak contained a small amount of radioactivity, and the VP 2 peak was virtually unlabeled. From Fig. 2b, c, and d (representing 4-, 6-, and 10-min pulses), it is evident that, as the

pulse time was increased, the relative amounts of radioactivity in VP 2 and VP 3 also increased, but VP 2 was always a lower percentage of its control than VP 3. VP 4 was not analyzed in these experiments.

To obtain quantitative values for the amount of each protein synthesized, the area under each peak was calculated and expressed as a percentage of its control. These results along with intermediate pulse times are tabulated in Table 1. A detailed discussion of the kinetics of appearance of each protein is presented below.

DISCUSSION

The purpose of performing these experiments was to determine the internal gene order of the part of the poliovirus genome coding for the four capsid proteins, a region which was previously shown to reside near the 5' end of the poliovirus RNA (16). The experiments reported here suggest that the internal order of this region from the 5' to the 3' end is VP 4 - VP 2 - VP 3 - VP 1. This order is identical to one proposed for encephalomyocarditis virus, a closely related picornavirus (R. Rueckert, *personal communication*).

The evidence for this suggestion comes from two different types of experiments. (i) After the addition of pactamycin there was a marked change in the relative amounts of the four viral coat proteins synthesized. When ^3H -leucine was added at 1 min 15 sec after the addition of the drug, synthesis of the protein VP 4 was most inhibited, followed in order by VP 2, VP 3, and VP 1. Furthermore, only VP 4 was inhibited in protein synthesized between 10 and 40 sec after the addition of pactamycin. From the analysis in our previous paper (16) and at the beginning of the present paper, a gene order of VP 4 - VP 2 - VP 3 - VP 1 is implied by these results. (ii) When the capsid proteins from precursor molecules labeled for a short time were analyzed, a differential labeling pattern was observed. After a 2-min pulse, virtually only VP 1 was labeled. Longer pulses were required to label VP 3 appreciably and even

TABLE 1. Experimental value of normalized ratio of each capsid protein (R_n)^a

Length of pulse (min)	R_2 (for VP 2)	R_3 (for VP 3)	R_1 (for VP 1)
2	0.018	0.11	1.00
3	0.11	0.37	1.00
4	0.27	0.58	1.00
5	0.47	0.68	1.00
6	0.60	0.73	1.00
10	0.81	0.86	1.00

^a As described in the text and Fig. 2, pulse-labeled virus proteins (VP) were compared to continuously labeled control virus. The areas under both the experimental and control peaks at each time were calculated and are expressed as a ratio.

longer pulses for VP 2. VP 4 was not analyzed in this study. Since the labeling periods in this experiment were terminated by the addition of emetine, a drug which freezes the movement of ribosomes on a messenger RNA, the 3'-proximal protein should be the first one to appear labeled among the molecules released from the polyribosomes during the labeling period, followed in turn by the proteins nearer the 5' end of the RNA. This experiment, therefore, also establishes a gene order of VP 2 - VP 3 - VP 1.

Because it has been previously demonstrated that the proteins VP 4 and VP 2 are cleavage products from the product VP 0, VP 0 would be expected to map in the same position as VP 4 and VP 2 (11). Unpublished experiments examining the proteins VP 0, VP 1, and VP 3 of procapsids synthesized in the presence of pactamycin confirm this expectation (D. Rekosh, *unpublished data*).

A summary of the genetic map for the major poliovirus proteins is shown in Fig. 3. The dashed lines and spaces indicate that it is not known whether the proteins are contiguous on the RNA or have "spaces" between them.

The amounts of three of the capsid proteins (VP 1, VP 2, VP 3) synthesized and released from polyribosomes after pulses of various length are

FIG. 2. Polyacrylamide gel electrophoresis of poliovirus capsid proteins synthesized after short pulses terminated by emetine addition. (a) At 3 hr after infection, cells were resuspended in Earle's saline plus 5% dialyzed horse serum, ^3H -leucine was added to 7 $\mu\text{Ci}/\text{ml}$, and 2 min later emetine was added to 100 $\mu\text{g}/\text{ml}$. The control was prepared as in Fig. 1b, and other procedures were as in Fig. 1a and 1b. Radioactivity was normalized to 100 in fraction 47, representing 1,206 counts/min of ^3H and 732 counts/min of ^{14}C . (b) Cells were treated as in (a) except that emetine was added 4 min after the addition of ^3H -leucine. Radioactivity was normalized to 100 in fraction 35 representing 2,240 counts/min of ^3H and 854 counts/min of ^{14}C . (c) Cells were treated as in (a) except that emetine was added 6 min after the addition of ^3H -leucine. Radioactivity was normalized to 100 in fraction 33 representing 1,870 counts/min of ^3H and 2,348 counts/min of ^{14}C . (d) Cells were treated as in (a), except that emetine was added 10 min after the addition of ^3H -leucine. Radioactivity was normalized to 100 in fraction 31, representing 1,946 counts/min of ^3H and 1,972 counts/min of ^{14}C . Symbols: Δ , ^3H -labeled experimental sample; $\bullet$, ^{14}C -labeled control.

FIG. 3. Genetic map of the major poliovirus proteins. It has been suggested that three proteins (NCVP 1, NCVP X, and NCVP 2) make up most, if not all of the poliovirus genome (2, 10, 12). NCVP 1 is cleaved to form VP 0, VP 1, and VP 3. VP 0 is further cleaved into VP 2 and VP 4 (2, 11). The genetic map of the three primary proteins was determined previously from pactamycin experiments (16). The position of NCVP X is somewhat tentative, but recent unpublished experiments of C. Cole confirm its position. The order of the virion proteins within NCVP 1 comes from experiments reported in this paper. The dashes and spaces between proteins indicate that all or some of the proteins may not be contiguous.

tabulated in Table 1. The data are expressed as the ratio of the protein synthesized during a pulse to the amount of protein synthesized during a continuous labeling period. Furthermore, the data have been normalized so that the ratio for VP 1 at each time is specified at 1.00. This allows us to compare directly the ratios of the viral proteins synthesized during each labeling period.

In the Appendix, we derive equations describing the theoretical kinetics of synthesis of four proteins derived from a single precursor molecule. The assumptions made in the derivation reflect experimental observations. The only assumptions made for which there are no supporting experimental data are that the capsid proteins are contiguous and the ribosomes, in the polyribosomes, are regularly spaced along the messenger RNA. The values of the theoretical normalized ratios, for different pulse times, are tabulated in Table 2.

Figure 4 shows a plot of the experimental normalized ratios from Table 1 compared to the theoretical equations describing the appearance of each protein for a given rate of chain elongation. The values for the size of each viral protein (L_n) have previously been determined by SDS-acrylamide gel analysis, and they are given in the legend of Fig. 4. It is seen (Fig. 4) that, at a rate of chain elongation of 205 amino acids per min, the theoretical equation fits the data for the appearance of VP 3 very well. The slightly higher rate needed to fit the theoretical curve to the VP 2 data is probably the result of a systematic overestimation of VP 2 in the experimental samples. The ratios (R_n) for VP 2 are hard to determine precisely because the protein migrates between the other two and does not form a completely separate peak.

The value of 205 to 225 amino acids per min observed as a rate of chain elongation is almost identical to a rate of 210 to 250 amino acids per min observed in the synthesis of protein by encephalocarditis virus, a similar picornavirus (R. Rueckert, *personal communication*). Both of these

rates, however, are considerably slower than those of 600 and 400 amino acids per min observed for the alpha and beta chains of hemoglobin in rabbit reticulocytes (9) and 900 amino acids per min observed for the synthesis of β -galactosidase in *Escherichia coli* (13). These differences cannot be reconciled until more is known about the rates of translation of the same messenger RNA in the different cell types.

Since the length of the poliovirus genome is 2.5×10^6 daltons (5), it is implied from the rate of chain elongation obtained in these experiments and the mechanism of synthesis of the poliovirus proteins that it takes 11 to 12 min for a single ribosome to traverse the whole RNA molecule. This is consistent with the estimate of 10 min for formation of a full-size viral polyribosome from a newly synthesized molecule of viral RNA (8). Furthermore, a pulse of radioactivity as short as 2 min, followed by inhibition of synthesis with emetine, is all that is needed to label some of the virion proteins. These proteins are derived from NCVP 1 which maps at the 5' end of the molecule. Given these facts, one concludes that NCVP 1 must be released from the polyribosome independently of the release of protein from the 3' end of the messenger RNA. This supports a model of nascent cleavage of the polypeptide chain as proposed previously (1, 4, 10).

APPENDIX

Derivations of equations describing the appearance of each capsid protein as a function of pulse time. The equations derived in this section describe a theoretical situation approximating the experiments with emetine described in the text.

The assumptions made for the derivation are as follows. (i) The gene order of the four capsid proteins on the RNA is (5' $\rightarrow$ 3') VP 4 - VP 2 - VP 3 - VP 1. (ii) The precursor (NCVP 1) is initially synthesized, released intact from the polyribosome, and later cleaved into the capsid proteins (4, 10, 12). (iii) The four capsid proteins are con-

TABLE 2. Theoretical value of normalized ratio (R_n)^a

Length of pulse	R_1	R_2	R_3	R_4
$0 < t \leq \frac{L_1}{K}$	1.00	0	0	0
$\frac{L_1}{K} \leq t \leq \frac{L_1 + L_3}{K}$	1.00	0	$\frac{(Kt - L_1)^2}{L_3(2Kt - L_1)}$	0
$\frac{L_1 + L_3}{K} \leq t \leq \frac{L_1 + L_3 + L_2}{K}$	1.00	$\frac{(Kt - L_1 - L_3)^2}{L_2(2Kt - L_1)}$	$\frac{2Kt - 2L_1 - L_3}{2Kt - L_1}$	0
$\frac{L_1 + L_3 + L_2}{K} \leq t \leq \frac{L_1 + L_3 + L_2 + L_4}{K}$	1.00	$\frac{2Kt - 2L_1 - L_3}{2Kt - L_1}$	$\frac{2Kt - 2L_1 - L_3}{2Kt - L_1}$	$\frac{(Kt - L_1 - L_3 - L_2)^2}{L_4(2Kt - L_1)}$
$\frac{L_1 + L_3 + L_2 + L_4}{K} \leq t \leq T$	1.00	$\frac{2Kt - 2L_1 - L_3}{2Kt - L_1}$	$\frac{2Kt - 2L_1 - L_3}{2Kt - L_1}$	$\frac{2Kt - 2L_1 - 2L_3 - 2L_2 - L_4}{2Kt - L_1}$

^a As derived in the appendix, the equations expressing the normalized ratio for each capsid protein were integrated and tabulated as a function of pulse length.

tiguous and, if any part of NCVP 1 is *not* represented in the capsid proteins, it is at the ends of the molecule. (iv) When protein synthesis is inhibited by addition of emetine after a short pulse, inhibition occurs instantaneously and the nascent chains remaining attached to the polyribosomes (7) do not get processed into capsid proteins. (v) RNA is translated 5' → 3' into protein (17). (vi) Ribosomes are equally spaced along the RNA.

Consider the NCVP 1 region as starting at its 3' end so distance is measured in numbers of amino acids from the 3' end of VP 1. Let this distance variable be X . Then, after a pulse of radioactivity of time t , followed by an instantaneous inhibition with emetine, the number of ribosomes on a single polyribosome passing the 3' end of the VP 1 region, having a completed protein with radioactivity at any point X is given by $r(Kt - X)$ for $Kt \geq X$; 0 for $Kt \leq X$ where r is the number of ribosomes per amino acid codon (three nucleotides) on the region of the RNA coding for NCVP 1 and K is the rate of chain elongation.

As each ribosome passes the terminus of NCVP 1, the nascent protein is cleaved off so the number of ribosomes becomes equivalent to the number of released NCVP 1 molecules having radioactivity at point X . Therefore, the total number of molecules having radioactivity in the small region dX equals $dA = qr(Kt - X)dX$ for $Kt \geq X$; $dA = 0$ for $Kt \leq X$ where q is the ratio of radioactive amino acids to nonradioactive amino acids in the region dX .

Let L_n = the length of VP n
for $n = 1, 2, 3$, or 4.

Let A_n = the amount of radioactivity in protein VP n for $n = 1, 2, 3$, or 4.

Let T = a long labeling time.

Then, for $0 < t \leq \frac{a_n}{K}$; $A_n = 0$

for $\frac{a_n}{K} \leq t \leq \frac{b_n}{K}$; $A_n = qr \int_{a_n}^{Kt} (Kt - x) dX$

for $\frac{b_n}{K} \leq t < T$; $A_n = qr \int_{a_n}^{b_n} (Kt - x) dX$

for $t = T$; $A_n = A_n^* = qrKtL_n$

with a_n and b_n defined below.

When

$n = 1$ $a_n = 0$; $b_n = L_1$

$n = 3$ $a_n = L_1$; $b_n = L_1 + L_3$

FIG. 4. Theoretical and experimental normalized ratios of each capsid protein synthesized during short pulses of various length. The data tabulated in Table 1 are plotted as a function of length of pulse in minutes. Symbols: (○), the normalized ratio R_3 for VP 3; (●), the normalized ratio R_2 for VP 2. The theoretical values of each normalized ratio, as derived in the appendix and given in Table 2, are superimposed on the data. The solid lines (—) indicate the curve for VP 3, the dashed lines (---) indicate the curve for VP 2. The length assumed for each protein in amino acid residues was VP 1 = 318, VP 2 = 254, VP 3 = 209 using the molecular weight determinations of reference (10) and an average molecular weight per amino acid of 110. The equations describing each curve are:

$$\text{for VP 3 } R_3 = \frac{Kt - 423}{Kt - 159} \quad Kt \geq 527; R_3 = \frac{(Kt - 318)^2}{418(Kt - 159)} \quad 318 \leq Kt \leq 527$$

$$\text{for VP 2 } R_2 = \frac{Kt - 654}{Kt - 159} \quad Kt \geq 781; R_2 = \frac{(Kt - 527)^2}{508(Kt - 159)} \quad 527 \leq Kt \leq 781$$

where K is the rate of chain elongation in amino acids per minute and t is the length of the pulse in minutes.

$$n = 2 \quad a_n = L_1 + L_3 \quad ; \quad b_n = L_1 + L_3 + L_2$$

$$n = 4 \quad a_n = L_1 + L_3 + L_2 ; \\ b_n = L_1 + L_3 + L_2 + L_4$$

given that the gene order ($5' \rightarrow 3'$) is VP 4 - VP 2 - VP 3 - VP 1. One next divides each A_n by each A_n^* and normalizes by dividing A_n/A_n^* by A_1/A_1^* to obtain the normalized ratios which are tabulated in Table 2.

ACKNOWLEDGMENTS

I thank Robert Taber for his collaboration on the original pactamycin experiments previously reported and for helpful discussions which led to some of the ideas and experiments reported here. I also thank David Baltimore for his advice, helpful discussions, and critical reading of the manuscript. Roland Rueckert and Byron Butterworth are thanked for making their results known to me before publication.

The equations presented in the appendix were derived with the help of Paul Calvert and Stanley Perlman.

This work was supported by Public Health Service grant AI-08388 from the National Institute of Allergy and Infectious Diseases to David Baltimore.

LITERATURE CITED

- Baltimore, D. 1971. Expression of animal virus genomes. *Bacteriol. Rev.* 35:235-241.
- Baltimore, D. 1971. Polio is not dead, p. 1-12. *In* M. Pollard (ed.), *From molecules to man: perspectives in virology*, vol. VII. Academic Press Inc., New York.
- Baltimore, D., M. Girard, and J. E. Darnell. 1966. Aspects of the synthesis of poliovirus RNA and the formation of virus particles. *Virology* 29:179-189.
- Baltimore, D., M. F. Jacobson, J. Asso, and A. S. Huang. 1969. The formation of poliovirus proteins. *Cold Spring Harbor Symp. Quant. Biol.* 34:741-746.
- Granboulan, N., and M. Girard. 1969. Molecular weight of poliovirus ribonucleic acid. *J. Virol.* 4:475-479.
- Grollman, A. P. 1966. Structural basis for inhibition of protein synthesis by emetine and cycloheximide based on an analogy between ipecac alkaloids and glutarimide antibiotics. *Proc. Nat. Acad. Sci. U.S.A.* 56:1867-1874.
- Grollman, A. P. 1968. Inhibitors of protein biosynthesis. V. Effects of emetine on protein and nucleic acid biosynthesis in HeLa cells. *J. Biol. Chem.* 243:4089-4094.
- Huang, A. S., and D. Baltimore. 1970. Initiation of polyribosomes formation in poliovirus-infected HeLa cells. *J. Mol. Biol.* 47:275-291.
- Hunt, T., T. Hunter, and A. Munro. 1969. Control of haemoglobin synthesis: rate of translation by messenger RNA for the α and β chains. *J. Mol. Biol.* 43:123-133.
- Jacobson, M. F., J. Asso, and D. Baltimore. 1970. Further evidence on the formation of poliovirus proteins. *J. Mol. Biol.* 49:657-669.
- Jacobson, M. F., and D. Baltimore. 1968. Morphogenesis of poliovirus. I. Association of the viral RNA with coat protein. *J. Mol. Biol.* 33:369.

12. Jacobson, M. F., and D. Baltimore. 1968. Polypeptide cleavages in the formation of poliovirus proteins. *Proc. Nat. Acad. Sci. U.S.A.* 61:77-84.
13. Lacroute, F. and G. S. Stent. 1968. Peptide chain growth of β -galactosidase in *Escherichia coli*. *J. Mol. Biol.* 35:165-173.
14. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-682.
15. Perlman, S., and S. Penman. 1970. Mitochondrial protein synthesis: resistance to emetine and response to RNA synthesis inhibitors. *Biochem. Biophys. Res. Commun.* 40:941-948.
16. Taber, R., D. Rekosh, and D. Baltimore. 1971. Effect of pactamycin on synthesis of poliovirus proteins: a method for genetic mapping. *J. Virol.* 8:395-401.
17. Watson, J. D. 1970. *Molecular biology of the gene*. W. A. Benjamin, Inc., New York.