Irreversible Inactivation by 2-Amino-4,6-Dichloropyrimidine of Certain Structural Proteins of Poliovirus

Previous work from this laboratory demonstrated that Poliovirus 1 morphogenesis is inhibited by 2-amino-4,6-dichloropyrimidine (Py 11)[1,2]. It is known that the structural proteins of poliovirus originate from a common precursor (NCVP 1). At first, a cleavage of this molecule gives place to the subunits VP0, VP1, VP3 which are organized in the procapsid structure. A successive cleavage of VP0 into VP2 and VP4 occurs upon assembly of virus RNA into the procapsid structure (provirion stage), thus leading to the normal set of virion proteins (VP1, VP2, VP3, VP4) typical of the infectious poliovirus particles[3–5]. The effect of Py 11 on these steps of virus maturation was investigated.

Materials and methods. Experiments were carried out in HEp 2 cell monolayers (American Type Culture Collection) which were infected at 4 °C for 1 h with Poliovirus 1 Brunenders (5×10^7 cells per sample, 100 plaque forming units-PFU-per cell) and incubated at 37 °C in aminoacid-free Eagle's MEM (AFE, pH 7.3) supplemented with 2% calf serum and 2 μg/ml of actinomycin D (AMD, Merck). Py 11 was added (100 μg/ml) to the cultures at time 0 after infection. The inhibitory action was, when due, reversed by incubating cell cultures in fresh, drug-free AFE, containing 50 μg/ml of L-glutamine and L-cysteine[2]. Between 2.30 and 3 h postinfection cells were labelled with ^{3}H-leucine (Amersham, 14.5 Ci/mMol, 2 μCi/ml) and, when due, chased with 100 μg/ml of L-leucine. Cells were then scraped from the glass and pelletted at 3,000 rpm for 5 min. Extracts from these cells were used to study both the organization of virus particles, according to the sucrose gradient technique of JACOBSON and BALTIMORE[3] and FERNANDEZ-TOMAS and BALTIMORE[4], and the formation of virus protein subunits, according to the electrophoretical method of SUMMERS et al.[5]. In the latter case, ^{14}C-leucine labelled proteins, obtained from purified procapsids and complete particles of poliovirus, were allowed to run together with the cell extracts, to serve as a reference point for structural proteins. More details are given in the Figures.

Results. The total pattern of virus proteins synthesized in the absence or in the presence of Py 11 was, at first,

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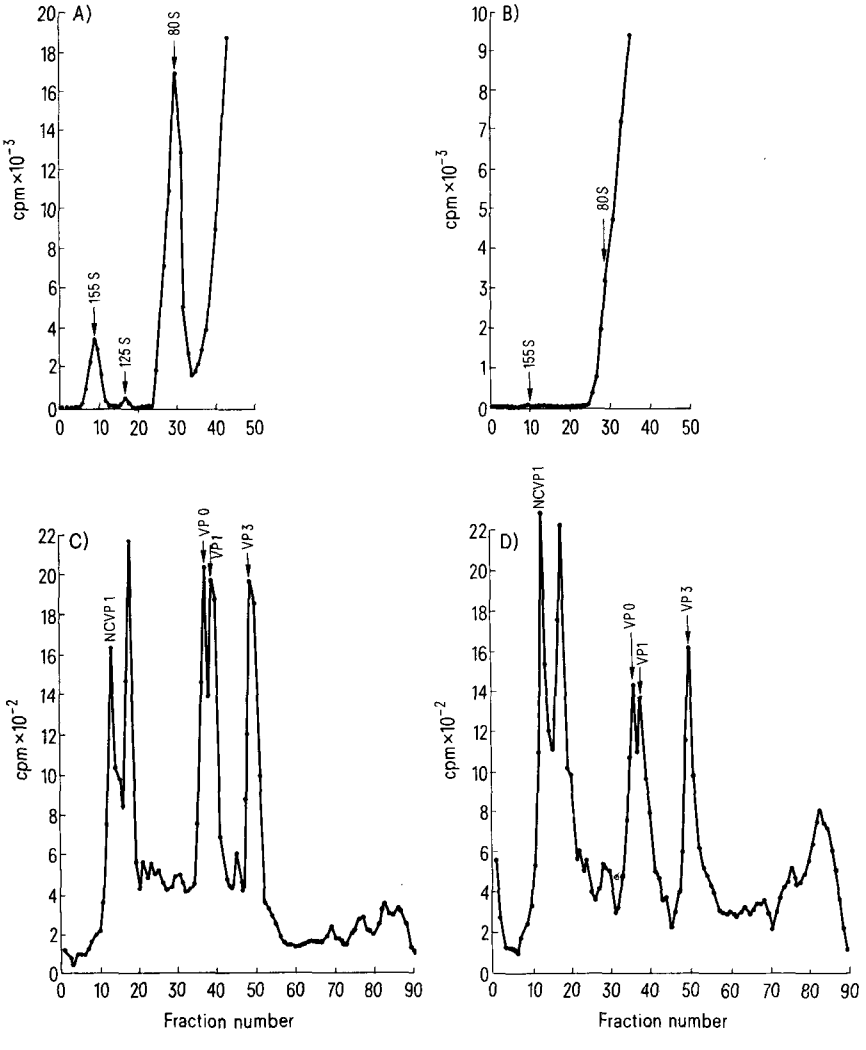

Fig. 1. Effect of Py 11 on structural poliovirus proteins, evaluated at the procapsid stage of maturation. (A) and (B) sucrose gradients of extracts from untreated (AFE) and Py 11 treated cells (15–30% sucrose in RSB, 24,000 rpm for 3.30 h at 20 °C in Spinco SW 25.2). (C) and (D) acrylamide gel electrophoretic profiles of extracts from untreated (AFE) and Py 11 treated cells (3 volts/cm for 12 h at 20 °C).

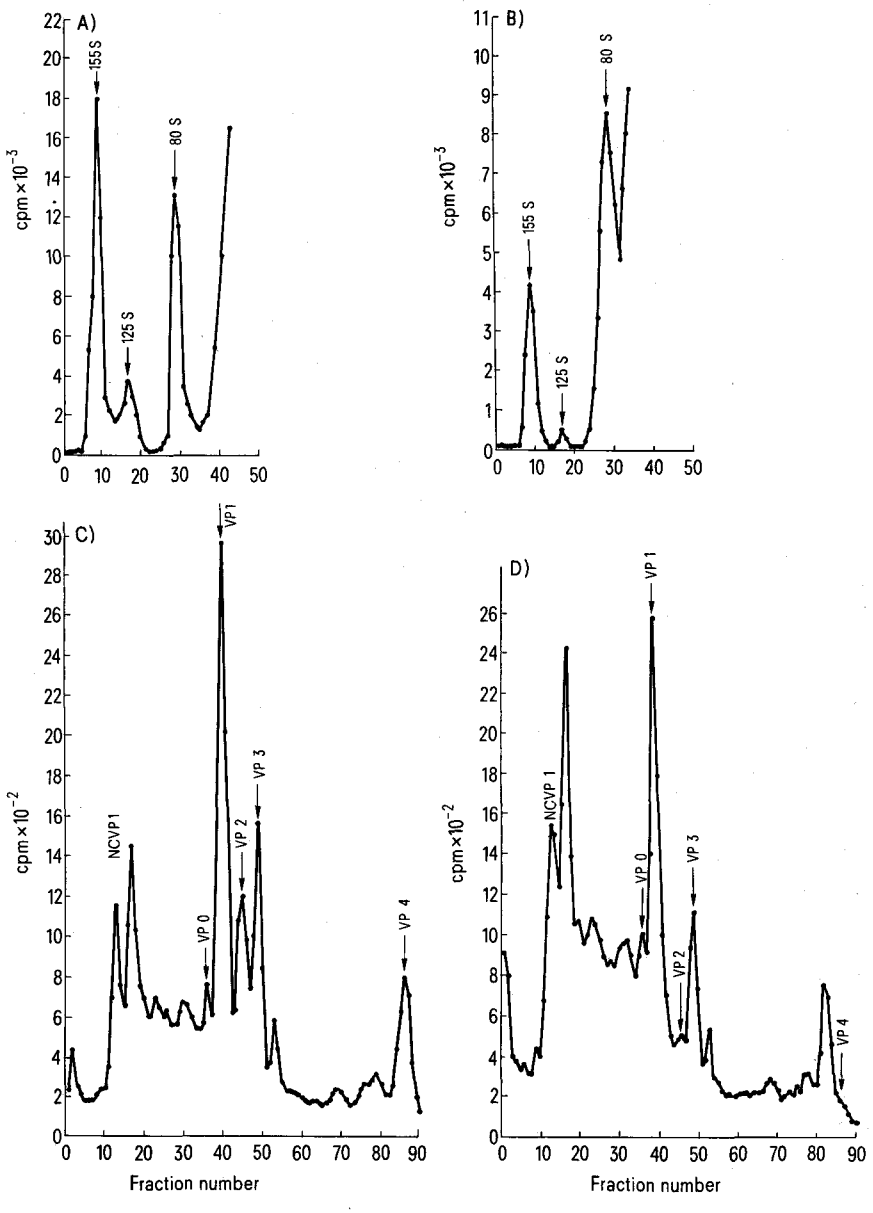

Fig. 2. Effect of Py 11 on structural poliovirus proteins, evaluated at the stage of complete virus maturation. (A) and (B) sucrose gradients of extracts from untreated (AFE) and Py 11 pretreated cells. (C) and (D) acrylamide gel electrophoretic profiles of extracts from untreated (AFE) and Py 11 pretreated cells.

analyzed soon after the labelling period, i.e. 3 h post-infection, at a time when structural proteins are mostly at the procapsid stage. As shown in Figure 1, under these conditions Py 11 only produces a slight decrease in the amount of VP0, VP1, VP3, an increase in the amount of NCVP1 and, in addition, an accumulation of low molecular weight proteins in the right part of the electrophoretic profile. However, very few, if any, of the structural proteins synthesized in the presence of Py 11 become part of procapsid, provirion and virion structures (80 S, 125 S and 155 S in sucrose gradient, respectively).

Experiments were then repeated by analyzing virus proteins, labelled as above, after a 2-h chase in drug-free medium, thus creating conditions under which virus RNA is incorporated into infectious virus both in untreated and in Py 11 pretreated cells[1,2]. Data in Figure 2 show that virus proteins synthesized in the presence of Py 11 become part of procapsid, provirion and mature virus particles when the drug is removed from the medium. However, as shown by the electrophoretic analysis, neither VP2 nor VP4 result among these proteins.

Taking into account that VP0 cleavage follows the organization of procapsid structures, the data obtained from the experiments reported here suggest that Py 11 irreversibly impairs the ability of VP0 to participate in the formation of capsid precursors thus preventing the cleavage of VP0 into VP2 and VP4 and that a new synthesis of VP0 in a drug-free medium is needed for the organization of procapsid and of mature virus particles. Electrophoretic studies of virus proteins obtained from purified virus particles are in progress to verify this hypothesis.

Résumé. L'effet inhibiteur de la 2-amino-4, 6-dichloropyrimidine (Py 11) sur la réplication du poliovirus est très probablement dûe à l'inactivation de certaines proteines structurelles du virus.

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