PURIFICATION AND CHARACTERIZATION OF DEFECTIVE INTERFERING PARTICLES OF SENDAI VIRUS

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Sokol *et al.* (2) have demonstrated that defective interfering particles (DIP) of Sendai (murine parainfluenza type 1) virus can be generated by the von Magnus (3) method of "incomplete" influenza virus production, i.e. by serial passages of undiluted infected allantoic fluids (IAF) in chick embryos (CE). Nevertheless, the problem of separation of Sendai DIP from IAF host components (HC) remained unsolved. The aim of the present work has been the elaboration of a simple and efficient purification procedure for Sendai DIP and their characterization.

Fresh IAF used for purification of DIP, prepared by serial passages of undiluted Sendai virus IAF in 9 to 11-day-old CE, had an EID_{50} to HAU ratio equal to 10^{17}. In contrast, IAF prepared by serial passages of 10^4 times diluted IAF in CE, containing practically only standard particles (SP) of Sendai virus, had an EID_{50} to HAU ratio equal to 10^5. Thus, the number of DIP in IAF used for their purification exceeded more than one thousand times the number of SP. From this IAF the DIP were concentrated in phosphate buffered saline pH 7.2 (PBS) by differential centrifugations. The resulting DIP suspension (9.1 mg of protein per ml) was electrophoretically heterogenous, as examined by moving boundary electrophoresis at pH 7.2, in PBS (Antweiler's apparatus, Boskamp, F.R.G.). Also electron microscopic (EM) examination of negatively stained suspension (with PTA) revealed heavy contamination of DIP with HC. Attempts to separate DIP from HC by zonal rate or equilibrium centrifugations in gradients of sucrose failed, because of presence of HC in IAF having the same size and density as DIP. Therefore, adsorption of DIP to and elution from rooster erythrocytes treated with glutaraldehyde (GA-ER) according to Hosaka and Hosokawa (1) was performed. Briefly, to DIP suspension diluted to 10^4 HAU/ml with PBS and cooled to 4 °C, an equal volume of chilled 50% suspension of GA-ER in PBS was added and the virus was allowed to adsorb for 20 min at 4 °C. After washing with chilled PBS the virus was released in PBS by several elution steps (15 min each) at 37 °C. In an average experiment, the total yield of virus (as determined by HA activity) in pooled eluates concentrated by differential centrifugations was 66%. In parallel, SP were also purified in a similar way from IAF prepared by serial passages of 10^4 times diluted IAF in CE. Both, purified DIP and SP, showed in electrophoresis (at pH 7.2, in PBS) a single peak with shoulder, the latter being more pronounced after the purification procedure had been repeated, thus indicating its viral origin. The absence of HC in DIP and SP preparations was also confirmed by EM examination. Preliminary experiments revealed that a comparable purity was achieved when the virus had been adsorbed on GA-ER directly from IAT, without previous concentration by differential centrifugations. Than, the resulting purified preparation showed a single peak in electrophoresis, without shoulder.

As revealed by co-electrophoresis of DIP and SP, their mobilities (at pH 7.2 in PBS) were identical. As determined from the electron micrographs, the arithmetic means of the diameters (largest end-to-end distances) of DIP (294 particles measured) and of SP (136 particles) were 172 nm and 271 nm, respectively. Statistically evaluated by the Student's t-test, the difference between means was significant with probability higher than 99.9%. Previous comparative investigations on Sendai DIP and SP have been further extended to the studies of their pathogenicity (after i.n. and i.c. inoculations) and of their immunogenicity for mice.

References

1. Hosaka, Y., and Hosokawa, Y., *Intervirology* **8** : 1, 1977.
2. Sokol, F., Neurath, A. R., and Vilček, J., *Acta virol.* **8** : 59, 1964.
3. von Magnus, P., *Acta path. microbiol. scand.* **28** : 278, 1951.