Photoaffinity labeling of rotavirus VP1 with 8-azido-ATP: Identification of the viral RNA polymerase

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Rotavirus single-shelled particles have several enzymatic activities that are involved with the synthesis of capped mRNAs both in vivo and in vitro. Because single-shelled particles must be structurally intact to carry out transcription, it has proven to be difficult to identify the protein within such particles that possesses associated RNA polymerase activity. One approach for characterizing the function of the individual proteins within single-shelled particles is to use nucleotide analogs to specifically label those proteins, such as the viral RNA polymerase, that have affinity for nucleotides. In this study, 8-azido-ATP (azido-ATP), a photoreactable nucleotide analog, was used to identify the viral RNA polymerase on the basis of the ability of the analog to inhibit transcription activity associated with rotavirus particles on exposure to UV light. When single-shelled particles were treated with UV light in the presence of [?-32P]azido-ATP, the structural protein VP1 became radiola