Stimulation of Transcription by \( S\)-Adenosyl-L-Homocysteine and Virion-encapsidated Methyl Donor in Spring Viraemia of Carp Virus

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SUMMARY

\( S\)-Adenosyl-L-methionine (SAM), a methyl donor, and its analogue \( S\)-adenosyl-L-homocysteine (SAH), an inhibitor of methylation, stimulate the activity of spring viraemia of carp virus (SVCV) virion transcriptase. The stimulation observed for SVCV is analogous to that observed previously (Furuichi, 1974, 1978) for a totally unrelated virus, cytoplasmic polyhedrosis virus (CPV). In the absence of exogenous SAM, RNA with 5'-methylated termini (presumptive GpppAmpAp) was produced, indicating that SVCV has an endogenous methyl donor. Significantly less methylated termini were produced when SVCV nucleocapsids were used to prime \textit{in vitro} transcription reactions, suggesting that the majority of the endogenous methyl donor is not associated with the nucleocapsid. Partial removal of endogenous methyl donor by preparing nucleocapsids did not have any effect on the degree of stimulation by exogenous SAM or SAH. We conclude from this study that SAH has two effects on SVCV transcription, inhibition of methylation and stimulation of transcription.

Spring viraemia of carp virus (SVCV) is a rhabdovirus structurally similar to vesicular stomatitis virus (VSV) (Bishop & Smith, 1977). We have recently shown that although these two viruses are serologically distinct, they can synthesize mRNA species with similar 5'-termini either \textit{in vitro} (Gupta \textit{et al.}, 1979), or \textit{in vivo} (P. Roy & K. C. Gupta, unpublished results).

Previously, it was reported (Roy & Clewley, 1978) that \( S\)-adenosyl-L-methionine (SAM) stimulated the \textit{in vitro} transcription activity of SVCV by two- to threefold. This is analogous to the situation reported for CPV in which almost no transcription was obtained in the absence of SAM (Furuichi, 1974). Since SAM stimulated transcription (Roy & Clewley, 1978) and our previous studies suggested the presence of an endogenous methyl donor, it was of interest to determine whether any relationship existed between endogenous methyl donor and stimulation of transcription by exogenous SAM. Furuichi (1978) recently reported that \( S\)-adenosyl-L-homocysteine (SAH), an inhibitor of methylation, also activates cytoplasmic polyhedrosis virus (CPV) transcription. In this communication we show that an endogenous methyl donor is present in SVCV virions and that the SVCV virion transcriptase activity is stimulated by either SAM or SAH.

The addition of exogenous SAM to SVCV \textit{in vitro} reaction mixtures has been shown to stimulate SVCV transcription by two- to threefold. The stimulation is significant even at relatively low concentrations (50 \( \mu \text{M} \)) of SAM (Fig. 1\( a \)). At higher concentrations the stimulation gradually increases and then reaches a maximum at about 800 \( \mu \text{M} \)-SAM. It is only at very high concentrations of SAM (3-2 mM) that there is a slight reduction in this stimulation. SAH was also found to stimulate SVCV transcription (Fig. 1\( a \)). The dose-response curve observed for SAH was essentially similar to that obtained for SAM. Fig. 1\( b, c \) shows the kinetics of stimulation obtained in the presence of 800 \( \mu \text{M} \)-SAM, or 1-2 mM-SAHA respectively. A distinct lag (15 to 30 min) was observed before the maximum stimulation was achieved for reactions containing either SAM or SAH. Preincubating the complete reaction mixtures on ice for 30 min did not eliminate the lag. The dose-response curves and kinetics of stimulation suggest that the sites of action of SAM and SAH may be...
Fig. 1. Effect of different concentrations of SAM and SAH on transcription, and kinetics of transcription stimulation. Reaction constituents have been described previously (Gupta et al., 1979). (a) Effect of different concentrations of SAM (●) or SAH (O) on SVCV transcription. 125 μl reaction mixtures containing the appropriate concentrations of SAM (or SAH) were incubated at 22 °C for 1 h. α-32P-GTP was included in reaction mixtures to monitor RNA transcription. At 0 and 60 min 50 μl amounts were TCA-precipitated and the TCA-insoluble radioactivity recovered and counted. The 32P incorporation ranged from 2 × 10⁵ to 5 × 10⁵ ct/min. (b, c) Kinetics of (b) SAM stimulation and (c) SAH stimulation on SVCV transcription: O, SAM or SAH addition; O, control. For the SAM reaction 3H-UTP, and for SAH α-32P-GTP were used to monitor SVCV transcription. 330 μl reaction mixtures were incubated at 22 °C and at the indicated time intervals 50 μl amounts were withdrawn to assay the TCA-insoluble radioactivity. (d) Kinetics of SAM (0.8 mM) and SAH (1.2 mM) stimulation on reactions containing GppNHp (0.8 mM) instead of GTP: O, SAM addition; ▲, SAH addition; O, control. The conditions of incubation and assays of 32P radioactivity into transcripts are described in (a).

The same. Although a similar stimulation was obtained by either SAM or SAH in normal reaction conditions, in reactions where GTP was replaced by its analogue GppNHp, SAH was found to be more effective than SAM (Fig. 1d). We have observed that in the presence of both SAM and SAH, although a 2-6-fold stimulation of transcription occurred, the same degree of stimulation of the transcription by SAM (or SAH) also occurred when nucleocapsids were used instead of virions in the assays (data not shown). Although nucleocapsid preparations eliminated most of the endogenous methyl donor, no effect on degree of transcription stimulation by SAM or SAH was observed. Probably, the endogenous methyl donor is present in too small an amount to have any detectable effect on transcription.

To determine whether methylation occurred in the presence of SAH, transcription reactions were incubated in the presence of 3H-methyl-SAM (0-6 μm) with or without the addition of SAH (1-2 mM) and with α-32P-CTP. The stimulation of transcription was 1-2- and
Fig. 2. DEAE-cellulose column chromatography of RNase T2 digests of α-32P-ATP-labelled transcripts prepared under various conditions. RNase T2 digests were applied to columns with optical quantities of RNase A digests of chick rRNA (Gupta et al., 1979). Oligonucleotides were eluted by applying a 130 ml LiCl gradient (0 to 220 mM) in tris-EDTA-urea (10 mM:3 mM:7 M) buffer pH 8; identification of the oligonucleotides eluting from about -5 to -6 is based on our previous studies (Gupta et al., 1979; Gupta & Roy, 1980). (a) Elution profile of RNase T2 digest of transcripts prepared by virus in the absence of SAM: peak I, GpppAp plus pppAp; peak II, GpppAmpAp. (b) Transcripts synthesized by nucleoproteins in the absence of SAM: peaks I and II as for (a). (c) Transcripts synthesized by nucleoproteins in the presence of SAM: peak I, 7mGpppAmpAp plus pppAp; peak II, GpppAmpAp. (d) Transcripts synthesized by virus in the presence of SAH: peak I, GpppAp plus pppAp; peak II absent. Nucleocapsids were prepared by lysing virions in 2% Triton X-100 in the presence of 10 mM-tris-HCl pH 8 and 0.15 M-NaCl. The lysate was layered on 30% glycerol (in 0.15 M-NaCl, 10 mM-tris-HCl pH 8) in an SW50.1 centrifuge tube with a 0.5 ml glycerol cushion at the bottom. The tubes were centrifuged at 45 000 rev/min for 90 min and nucleocapsids on the glycerol cushion were recovered.

2.5-fold in the presence of 3H-methyl-SAM and -SAH respectively. However, when SAM and SAH were used in the same reaction mixture, methylation of transcripts was almost completely inhibited (97%), although a 2.6-fold stimulation of transcription was observed.

We have previously observed that in the absence of added SAM, transcripts are synthesized having a presumptive methylated cap structure (GpppAmp). To determine whether the endogenous virion methyl donor is associated with the viral nucleocapsids, we have compared the cap structures produced by SVCV particles to those obtained with purified nucleocapsids. Analyses of the ribonuclease T2 digests of α-32P-ATP-labelled transcripts by DEAE-cellulose column chromatography are presented in Fig. 2 for reactions
in which the template was SVCV particles or nucleocapsids. Fig. 2 (a) shows the analysis of termini synthesized by virions in the absence of SAM. The peak I nucleotides (Fig. 2a, pppAp and GpppAp, about 48% of total labelled termini) which eluted slightly earlier than the marker tetranucleotides (net charge -5) and peak II nucleotides (principally GpppAmpAp, about 52% of total labelled termini) which eluted between the marker tetra- and pentanucleotides (net charge -5 to -6), have been characterized previously (Gupta et al., 1979; Gupta & Roy, 1980). No attempt was made to recharacterize them.

Transcripts synthesized by purified SVCV nucleocapsids (Bishop 1977; Bishop et al., 1974) in the presence of α-32P-ATP and absence of SAM, were similarly analysed. The results presented in Fig. 2 (b) show that although both peak I (73% of label) and peak II (27% of label) nucleotides were present, much less peak II material was obtained. The peak I nucleotides were characterized by alkaline phosphatase digestion and found to contain both open (pppAp) and capped, non-methylated termini (GpppAp) in amounts similar to those found previously for SVCV particles (Gupta et al., 1979; Gupta & Roy, 1980; data not shown). The fact that some peak II material was also obtained from products made by SVCV nucleocapsids and shown by phosphatase analysis to contain capped structures of the type GpppAmpAp, suggests that either the nucleocapsid preparation contained small amounts of intact virions (which were difficult to exclude totally), or that some of the virion methyl donor was associated with the nucleocapsid. When transcripts were prepared by nucleocapsids in the presence of SAM and analysed by ribonuclease T2 digestion, both peak I (47%) and peak II (53%) nucleotides were recovered in amounts closely comparable to those obtained with SVCV particles (Fig. 2c). From these experiments we conclude that most of the SVCV particle endogenous methyl donor was solubilized when nucleocapsids were prepared. More rigorous attempts to remove the methyl donor from SVCV nucleocapsids have not been successful; rather they have led to the loss of all in vitro transcription capability.

When transcripts were synthesized by virions in the presence of SAH and analysed as above after RNase T2 digestion, the peak II material totally disappeared (Fig. 2d). These results are consistent with the conclusion that an endogenous methyl donor is present in purified virions.

Stimulation of transcription by SAM has been reported previously for CPV (Furuichi, 1974) and SVCV (Roy & Clewley, 1978). Recently, Furuichi (1978) reported an interesting observation that simulation is not methylation-dependent and can be observed in the presence of SAH, an inhibitor of methylation. The results presented in this communication provide further support for Furuichi’s observations. However, the stimulation observed for SVCV by SAM or SAH is not an all-or-nothing effect as in CPV, although it is significant (two- to threefold). The stimulatory effect of SAM or SAH may have some significance in providing an understanding of the procedures involved in transcription. However, at present it is not clear why SAM, or SAH, stimulates SVCV (or CPV) transcription. Possibly, SAM, or a chemically similar compound (e.g. SAH), may act as an allosteric stimulator for the viral RNA polymerase, or bind to the transcription initiation complex and thereby increase the affinity of the complex for the polymerase. SVCV reactions with SAM or SAH have a distinct lag phase, suggesting that their effects are not direct (for example, possibly they promote the rate of enzyme reinitiation). We have recently shown that in the presence of the GTP analogue, GppNHp, the efficiency of capping is considerably reduced (Gupta & Roy, 1980). Since in the presence of GppNHp we have also observed transcription stimulation by SAM or SAH, it seems likely that the involvement of SAM or SAH in transcription enhancement is independent of the mechanism of capping.

However, when VSV transcripts were synthesized in vitro in the presence of SAH and ascites cell extract, heterogeneous unmethylated giant poly(A)-containing transcripts were obtained (Rose et al., 1977). The investigators suggested a role of methylation on
polyadenylation of mRNAs. In contrast, we have synthesized message-length RNA species of SVCV in vitro in the presence of SAH (Gupta & Roy, 1980; K. C. Gupta & P. Roy, unpublished results). The discrepancy in the results from two viruses belonging to the same family (Rhabdoviridae) are as yet unexplained and need further investigation.

The presence of an endogenous methyl donor in SVCV particles raises the question of its origin. Transcripts made by SVCV nucleocapsids contain much less methylated termini, although when SAM was added they still had the capacity to produce them. These results suggest that much of the virion methyl donor is soluble, although we cannot exclude the possibility that some may be tightly nucleocapsid-associated.

In conclusion, our studies suggest that SAM has two effects on SVCV transcription: (i) methylation, and (ii) stimulation of the transcription rate. These two roles can be distinguished using SAH, which stimulates transcription but inhibits methylation. Purified virions have an encapsidated methyl donor, the exact chemical nature of which remains to be elucidated.

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REFERENCES


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