Inhibition of Newcastle Disease Virion Messenger RNA (guanine-7-)-Methyltransferase by Analogues of *S*-Adenosylhomocysteine†

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ABSTRACT: Structural analogues of *S*-adenosyl-L-homocysteine (AdoHcy), with modifications in the amino acid, base, or sugar portion of this molecule, were evaluated in vitro for their abilities to inhibit the *S*-adenosyl-L-methionine (AdoMet)-dependent transmethylation catalyzed by Newcastle disease virion mRNA (guanine-7-)-methyltransferase. Newcastle disease virions contain enzyme activities which in vitro synthesize a methylated-blocked mRNA with the 5′ terminus being m^{7}GpppG-. Kinetic inhibition constants were determined for those AdoHcy analogues that were found in preliminary studies to be good inhibitors of this virion mRNA (guanine-7-)-methyltransferase. *S*-Tubercidinyl-L-homocysteine (TubHcy), *S*-adenosyl-L-homocysteine sulfone (AdoHcy sulfone), *S*-adenosyl-L-homocysteine sulfoxide (AdoHcy sulfoxide), and *S*-N^6-methyladenosyl-L-homocysteine (N^6-methyl-AdoHcy) were found to be the most potent inhibitors of this transmethylation. Earlier studies have shown that TubHcy, like AdoHcy, is a general inhibitor of AdoMet-dependent methyltransferases. In contrast, AdoHcy sulfone, AdoHcy sulfoxide, and N^6-methyl-AdoHcy appear to be fairly specific inhibitors of this virion mRNA (guanine-7-)-methyltransferase. The significance of these data relative to the nature of AdoHcy binding site on this mRNA methyltransferase is discussed.

In the past several years, evidence has accumulated suggesting that many viral and eukaryotic mRNAs have the methylated structure, m^{7}GpppN$^{(m)}$, at the 5′ terminus (Shatkin, 1976). This methylated-blocked structure has been shown to be important for efficient translation and is required for binding the mRNA to the ribosome (e.g., Both et al., 1975a; Muthukrishnan, 1975a,b).

Both the sugar and base methylations at the 5′ terminus of mRNA are catalyzed by specific methyltransferases which require *S*-adenosyl-L-methionine (AdoMet)[1] as the methyl donor. A general feature of most AdoMet-dependent methyltransferases, including mRNA methylations, is the inhibition produced by the demethylated product, *S*-adenosyl-L-homocysteine (AdoHcy) (Borchardt, 1977). In recent years, several laboratories (Borchardt, 1977) have been attempting to elucidate the specificity of the enzymatic binding sites for AdoHcy by synthesizing and evaluating the inhibitory properties of AdoHcy analogues. Such information has been useful for the design of specific inhibitors of individual methyltransferases. Specific inhibitors of viral mRNA methyltransferases might be useful as antiviral agents since methylation is necessary for efficient translation of the viral mRNA and hence replication of the virus.

Newcastle disease virus, an avian paramyxovirus that replicates in the cytoplasm of infected cells, contains a ribonucleoprotein core inside the lipoprotein membrane which is able to synthesize methylated, blocked, and polyadenylated mRNA in vitro (Colonno and Stone, 1975, 1976b; Weiss and Bratt, 1974). The 5′ terminus of mRNA synthesized by virions in vitro has the structure m^{7}GpppG-, which lacks the 2′-O-methylated penultimate nucleotide as well as internal base methylations (Colonno and Stone, 1976a). The mRNA (guanine-7-)methyltransferase associated with the virus has previously been shown to be an AdoMet-dependent enzyme, which is sensitive to inhibition by AdoHcy (Colonno and Stone, 1975, 1976a). In order to characterize the AdoHcy binding site of Newcastle disease virion mRNA (guanine-7-)methyltransferase, various amino acid, base, or sugar modified analogues of AdoHcy were evaluated as inhibitors of this transmethylation. The present paper reports the results of this investigation.

Materials and Methods

Materials. *S*-Adenosyl-L-[*methyl*-^{3}H]methionine ([^{3}H]AdoMet, 11.43 Ci/mmol) was obtained from New England Nuclear, [5-^{3}H]uridine 5′-triphosphate ([5-^{3}H]UTP, 18 Ci/mmol) from Schwarz/Mann, and AdoHcy, ribonucleoside triphosphates, and AdoMet were from Sigma. The AdoHcy analogues (Table I) used in this study were synthesized according to previously published procedures as cited below: D-AdoHcy, AdoHcy sulfoxide, AdoHcy sulfone, AdoTpa, Ado-*N*-AcTpa, AdoTba, AdoTbMe ester, and Ado-*N*-AcHcy (Borchardt and Wu, 1974); GuaHcy, InoHcy, N^6-methyl-AdoHcy, 3-deaza-AdoHcy, N^6-methyl-3-deaza-AdoHcy, N^6-dimethyl-3-deaza-AdoHcy, UriHcy, and CytHcy (Borchardt et al., 1974); 2′-deoxy-AdoHcy and

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[1] Abbreviations used: Ado-*N*-AcHcy, *S*-adenosyl-L-*N*-acetylhomocysteine; Ado-*N*-AcTpa, *S*-adenosyl-3-*N*-acetylthiopropylamine; AdoCy, *S*-adenosyl-L-cysteine; AdoHcy, *S*-adenosyl-L-homocysteine; D-AdoHcy, *S*-adenosyl-D-homocysteine; AdoHcy sulfone, *S*-adenosyl-L-homocysteine sulfone; AdoHcy sulfoxide, *S*-adenosyl-L-homocysteine sulfoxide; AriHcy, *S*-aristeromycinyl-L-homocysteine; 2-aza-AdoHcy, *S*-2-azaadenosyl-L-homocysteine; 8-aza-AdoHcy, *S*-8-azaadenosyl-L-homocysteine; CytHcy, *S*-cytidyl-L-homocysteine; 3-deaza-AdoHcy, *S*-3-deazaadenosyl-L-homocysteine; AdoTba, *S*-adenosyl-4-thiobutyric acid; AdoTbMe ester, *S*-adenosyl-4-thiobutyrate methyl ester; AdoTpa, *S*-adenosyl-3-thiopropylamine; 2′-deoxy-AdoHcy, *S*-2′-deoxyadenosyl-L-homocysteine; 3′-deoxy-AdoHcy, *S*-3′-deoxyadenosyl-L-homocysteine; N^6-dimethyl-3-deaza-AdoHcy, *S*-N^6-dimethyl-3-deazaadenosyl-L-homocysteine; GuaHcy, *S*-guanosyl-L-homocysteine; InoHcy, *S*-inosyl-L-homocysteine; N^6-methyl-AdoHcy, *S*-N^6-methyladenosyl-L-homocysteine; N^6-methyl-3-deaza-AdoHcy, *S*-N^6-methyl-3-deazaadenosyl-L-homocysteine; TubHcy, *S*-tubercidinyl-L-homocysteine; UriHcy, *S*-uridyl-L-homocysteine; AdoMet, *S*-adenosyl-L-methionine; m^{7}G, 7-methylguanosine; N^m, 2′-*O*-methylnucleoside; Tris, tris(hydroxymethyl)-aminomethane; EDTA, ethylenediaminetetraacetic acid.

3′-deoxy-AdoHcy (Borchardt and Wu, 1975); AriHcy (Borchardt and Wu, 1976a); AdoCy, TubHcy, and 8-azaAdoHcy (Borchardt et al., 1976a). 2-Aza-AdoHcy was prepared by the condensation of 5′-chloro-5′-deoxy-2-azaadenosine and homocystine using the general procedure described earlier by our laboratory (Borchardt et al., 1976c).

Viral Purification. Newcastle disease virus, the Beaudette C strain (Granoff, 1959), was grown in 10-day-old embryonated chicken eggs at 37 °C for 36 h. Virions were purified from the allantoic fluid by differential, rate zonal, and isopycnic centrifugation as previously described (Colonno and Stone, 1975).

Nucleocapsid Isolation. Nucleocapsids (ribonucleoprotein cores) were isolated from purified virus as previously described (Colonno and Stone, 1976a). Briefly, the membrane and membrane-associated proteins were solubilized by treatment of intact virions with Triton X-100 and high salt. The nucleocapsids were then purified from solubilized material by isopycnic centrifugation in a D_2O–glycerol/D_2O–sucrose linear gradient.

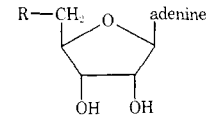
mRNA (guanine-7-)Methyltransferase Assay. The in vitro reaction mixture (100 μL) for assaying mRNA (guanine-7-)methyltransferase contained: 50 mM Tris-HCl (pH 7.8); 150 mM NaCl; 0.8 mM $MnCl_2$; 0.025% (v/v) Triton N-101; 0.7 mM UTP; 0.7 mM CTP; 0.7 mM GTP; and 0.7 mM ATP; 2.4 mM dithiothreitol; virions or nucleocapsids (15–25 μg of viral protein); variable amounts of [^{3}H]AdoMet (11.43 Ci/mmol); and variable amounts of SAH analogues. The [^{3}H]AdoMet was neutralized with dilute NH_4OH immediately before addition to the assay mixture. The assay was incubated at 32 °C for 4 h and terminated by the addition of 0.5 mL of buffer (50 mM Tris-HCl–1 mM EDTA, pH 7.4) and sodium dodecyl sulfate (0.5% final).

RNA Polymerase Assay. The assay for RNA polymerase was identical with that described above for the methyltransferase assay, except that [^{3}H]AdoMet and unlabeled UTP were replaced with unlabeled AdoMet and [5-^{3}H]UTP (0.23 mM, 0.163 Ci/mmol).

RNA Isolation. After termination of either the polymerase or the methyltransferase assays, the RNA was phenol–sodium dodecyl sulfate extracted and ethanol precipitated. The acid-insoluble radioactivity was determined as previously described (Colonno and Stone, 1975).

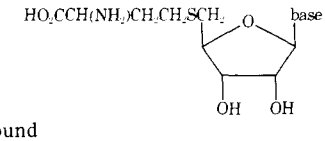
TABLE I: Structural Analogues of AdoHcy.

Amino Acid Modifications



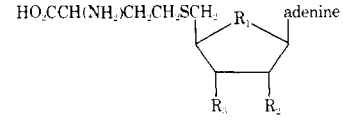
Compound	R
AdoHcy	$-SCH_2CH_2CH(NH_2)CO_2H$(L)
D-AdoHcy	$-SCH_2CH_2CH(NH_2)CO_2H$(D)
AdoHcy sulfone	$-S(\rightarrow O)CH_2CH_2CH(NH_2)CO_2H$(L)
AdoHcy sulfoxide	$-S(\overset{O}{\underset{O}{\diagup\diagdown}})CH_2CH_2CH(NH_2)CO_2H$(L)
Ado-*N*-AcHcy	$-SCH_2CH_2CH(NHAc)CO_2H$(L)
AdoTba	$-SCH_2CH_2CH_2CO_2H$
AdoTbMe ester	$-SCH_2CH_2CH_2CO_2CH_3$
AdoTpa	$-SCH_2CH_2CH_2NH_2$
Ado-*N*-AcTpa	$-SCH_2CH_2CH_2NHAc$
AdoCy	$-SCH_2CH(NH_2)CO_2H$(L)

Base Modifications



Compound	Base
TubHcy	7-Deazaadenine
N^6-Methyl-AdoHcy	N^6-Methyladenine
8-Aza-AdoHcy	8-Azaadenine
2-Aza-AdoHcy	2-Azaadenine
3-Deaza-AdoHcy	3-Deazaadenine
N^6-Methyl-3-deaza-AdoHcy	N^6-Methyl-3-deazaadenine
N^6-Dimethyl-3-deaza-AdoHcy	N^6-Dimethyl-3-deazaadenine
InoHcy	Hypoxanthine
GuaHcy	Guanine
UriHcy	Uracil
CytHcy	Cytosine

Sugar Modifications



Compound	R_1	R_2	R_3
AriHcy	CH_2	OH	OH
2′-Deoxy-AdoHcy	O	H	OH
3′-Deoxy-AdoHcy	O	OH	H

Results

Colonno and Stone (1975) have shown earlier that AdoHcy inhibits the transmethylation catalyzed by mRNA (guanine-7-)methyltransferase in Newcastle disease virions. This mRNA (guanine-7-)methyltransferase can be partially purified by solubilization of the intact virions with Triton X-100 and isopycnic centrifugation of the nucleocapsids on a D_2O–glycerol/D_2O–sucrose linear gradient (Colonno and Stone, 1976b). The mRNA (guanine-7-)methyltransferase associated with these purified nucleocapsids exhibits catalytic properties similar to the methylase activity in the intact virions, including sensitivity to inhibition by AdoHcy. Therefore, either the detergent-disrupted virions or purified nucleocapsids can be used as a source of mRNA (guanine-7-)methyltransferase for in vitro studies. Utilizing an in vitro assay system, which included [^{3}H]AdoMet to label the 5′ terminus (m^7GpppG–) of the newly synthesized mRNA, we determined the kinetics of AdoHcy inhibition of this mRNA (guanine-7-)methyltransferase. When AdoMet was the variable substrate, AdoHcy was shown to be a competitive inhibitor (K_i = 1.09 ± 0.14 μM). The competitive kinetics suggest that AdoHcy is competing for the AdoMet binding site on mRNA (guanine-7-)methyltransferase, a phenomenon consistent with other AdoMet-dependent methyltransferases (Borchardt, 1977).

In an effort to characterize the AdoHcy binding site on this mRNA (guanine-7-)methyltransferase, we have evaluated various analogues of AdoHcy for their abilities to inhibit this transmethylation. The AdoHcy analogues used in this study had modifications in either the amino acid, base, or sugar portions of this molecule (Table I). In preliminary experiments the effect of the AdoHcy analogues on [^{3}H]methyl incorporation from [^{3}H]AdoMet into newly synthesized mRNA was determined. The results listed in Table II are expressed as the percent inhibition of [^{3}H]methyl incorporation as compared with control experiments. In discussing their inhibitory activities, the analogues have been divided into three general classes: (a) amino acid modified derivatives; (b) base modified derivatives; and (c) sugar modified derivatives.

Of the amino acid modified analogues of AdoHcy, only those with modifications of the sulfur atom showed appreciable inhibitory activity (Table II). Both AdoHcy sulfone and

TABLE II: Inhibition of (guanine-7-)Methyltransferase and RNA Polymerase by AdoHcy Analogues.[a]

Compound	% inhibition of methyltransferase[b] 10 μM	% inhibition of methyltransferase[b] 100 μM	Inhibition constants[c] for methyltransferase (K_i ± SE, μM)	% inhibition of RNA polymerase[b] 100 μM
AdoHcy	71	94	1.09 ± 0.14	0
Amino acid modifications				
D-AdoHcy	0	58	26.0 ± 3.6	0
AdoHcy sulfone	47	90	2.01 ± 0.27	0
AdoHcy sulfoxide	63	95	1.15 ± 0.17	0
Ado-*N*-AcHcy	6	16	ND[d]	ND[d]
AdoTba	16	4	ND	ND
AdoTbMe ester	0	4	ND	ND
AdoTpa	0	0	ND	ND
Ado-*N*-AcTpa	4	9	ND	ND
AdoCy	0	0	ND	ND
Base modifications				
TubHcy	70	97	0.39 ± 0.06	0
N^6-Methyl-AdoHcy	48	88	2.08 ± 0.25	0
8-Aza-AdoHcy	27	84	9.22 ± 0.97	0
2-Aza-AdoHcy	7	21	ND	ND
3-Deaza-AdoHcy	3	49	26.3 ± 3.0	0
N^6-Methyl-3-deaza-AdoHcy	7	15	ND	ND
N^6-Dimethyl-3-deaza-AdoHcy	11	19	ND	ND
InoHcy	2	4	ND	ND
GuaHcy	11	0	ND	ND
UriHcy	8	1	ND	ND
CytHcy	0	0	ND	ND
Sugar modifications				
AriHcy	20	54	16.2 ± 2.0	0
2′-Deoxy-AdoHcy	10	48	26.0 ± 8.5	0
3′-Deoxy-AdoHcy	0	33	77.7 ± 30	0

[a] Purified Newcastle disease virus was assayed in vitro for mRNA (guanine-7-)methyltransferase and RNA polymerase as described under Materials and Methods. [b] In all cases AdoMet concentration was 0.54 μM, expressed as percent inhibition of control (no inhibitor added). Data represent averages of duplicate runs. AdoHcy analogue concentrations were 10 μM or 100 μM. [c] AdoMet was the variable substrate at concentrations varying between 0.27 and 2.70 μM. Each inhibitor showed linear competitive kinetics when plots of the reciprocal velocity vs. reciprocal SAM concentrations were made. Assays were done in duplicate. The inhibition constants were calculated as previously described (Cleland, 1967). [d] ND, not determined.

AdoHcy sulfoxide were potent inhibitors of mRNA (guanine-7-)methyltransferase. In contrast, this methyltransferase exhibited very high specificity for the other structural features of the homocysteine portion of AdoHcy. The structural features of primary importance in binding include: (1) the chirality of the amino acid asymmetric carbon, since D-AdoHcy exhibited reduced activity; (2) the terminal amino group, since Ado-*N*-AcHcy, AdoTba, and Ado-*N*-AcTpa were weak inhibitors; (3) the terminal carboxyl group, since AdoTpa and AdoTbMe ester were also weak inhibitors; and (4) the three-carbon distance between the sulfur atom and the terminal amino and carboxyl groups, since AdoHcy exhibited no inhibitory activity.

Of the base modified analogues, TubHcy, N^6-methyl-AdoHcy, and 8-aza-AdoHcy were potent inhibitors of mRNA (guanine-7-)methyltransferase. In sharp contrast, however, 2-aza-AdoHcy and 3-deaza-AdoHcy were only weak inhibitors. These results would suggest that some minor changes in selected positions on the adenine ring of AdoHcy can be tolerated in the enzymatic binding process. However, more drastic changes such as replacement of the adenine moiety of AdoHcy with a pyrimidine base (e.g., CtyHcy or UriHcy) or purine bases, which lack a 6-amino group (e.g., GuaHcy, InoHcy), result in complete loss of inhibitory activity. Thus the presence of a purine base containing a 6-amino group appears to be a minimum requirement for the binding of AdoHcy to this mRNA methyltransferase.

The marginal inhibitory activities (Table II) of the sugar modified analogues (AriHcy, 2′-deoxy-AdoHcy, 3′-deoxy-AdoHcy) indicate that the general features of the ribofuranose moiety, especially the 3′-hydroxy group, are necessary for maximum binding to this mRNA (guanine-7-)methyltransferase of Newcastle disease virus.

In order to ensure that the observed inhibition of mRNA (guanine-7-)methyltransferase was not due to de facto inhibition of RNA synthesis, the effects of AdoHcy, if any, on RNA polymerase were investigated. The effect of increasing concentration of AdoHcy on the methylation of mRNA was measured by determining the incorporation of [^{3}H]methyl from [^{3}H]AdoMet, whereas, the effect of the AdoHcy on RNA synthesis was measured by determining the incorporation of ^{3}H label from [5-^{3}H]UTP. While methylation was progressively inhibited by increasing concentrations of AdoHcy from 0 to 100 μM, RNA synthesis was not affected. In Table II we have also shown the effects of the AdoHcy analogues on RNA synthesis. None of the compounds tested showed any inhibitory effects on RNA synthesis.

For analogues showing inhibitory activity against mRNA (guanine-7-)methyltransferase in the preliminary studies, kinetic experiments were performed and inhibition constants (K_i) were calculated (Table II). With each of the active AdoHcy analogues competitive kinetic patterns, similar to that observed for AdoHcy, were observed when AdoMet was the variable substrate. For the sake of comparison, the K_m value

for AdoMet was calculated to be 0.119 ± 0.017 μM. Of the AdoHcy analogues evaluated, TubHcy was the best inhibitor, exhibiting an inhibition constant (K_i = 0.39 ± 0.06 μM) substantially lower than the natural inhibitor, AdoHcy (K_i = 1.09 ± 0.14 μM). AdoHcy sulfoxide, AdoHcy sulfone, and N^6-methyl-AdoHcy had inhibition constants similar to AdoHcy, whereas 8-aza-AdoHcy, AriHcy, 2′-deoxy-AdoHcy, D-AdoHcy, and 3-deaza-AdoHcy exhibited inhibition constants at least an order of magnitude greater than that of the parent compound.

Discussion

A general characteristic of AdoMet-dependent enzymes is their sensitivity to inhibition by the demethylated product, AdoHcy. Several laboratories (Borchardt, 1977) have attempted to take advantage of the inhibitory effects of AdoHcy for the design of specific inhibitors of AdoMet-dependent transmethylations. By systematically altering the chemical structure of AdoHcy and evaluating these analogues as inhibitors of a spectrum of physiologically important methyltransferases, information is now available concerning differences in the enzymatic binding requirements for AdoHcy. Earlier studies from our laboratory (Borchardt, 1975, 1976; Borchardt and Wu, 1974, 1975, 1976a,b; Borchardt et al., 1974, 1976a–c) have focused on the synthesis of AdoHcy and AdoMet analogues, and their evaluation as inhibitors of small molecule transmethylations (e.g., catechol-*O*-methyltransferase, phenylethanolamine-*N*-methyltransferase, histamine-*N*-methyltransferase, hydroxyindole-*O*-methyltransferase, and indoleethylamine-*N*-methyltransferase). As an extension of these studies we have reported here the effects of AdoHcy analogues on the transmethylation involved in the synthesis of the capped–methylated structures present at the 5′ terminus of viral and eukaryotic mRNAs. These capped–methylated structures at the 5′ terminus of mRNAs have been shown to be important for efficient translation and binding of the mRNA to the 40S subunit of the ribosome (Both et al., 1975a,b; Muthukrishnan et al., 1975a,b). Thus, specific inhibition of the enzymes responsible for methylation of the capped structure of viral mrna should yield a poorly translatable messenger, which would hinder or possibly halt the replication of the virus. As a result, inhibitors of these transmethylation processes might function as antiviral agents.

By evaluating a series of AdoHcy analogues as inhibitors of Newcastle disease virion mRNA (guanine-7-)methyltransferase, we have been able to delineate the structural features of AdoHcy needed for maximum binding to this methyltransferase. For example, it can be concluded from the data that the chirality of the amino acid asymmetric carbon of AdoHcy is not absolutely critical for binding to this (guanine-7-)methyltransferase, since D-AdoHcy exhibited inhibitory activity. D-AdoHcy was shown previously to be a more potent inhibitor of *Escherichia coli* tRNA methyltransferase (Hildesheim et al., 1973) and histamine-*N*-methyltransferase (Borchardt and Wu, 1974). However, D-AdoHcy had little or no inhibitory activity against other small molecule methylations (Borchardt and Wu, 1974; Borchardt, 1975). Also of particular interest were AdoHcy sulfone and AdoHcy sulfoxide, because of their potent inhibitory effects on this mRNA (guanine-7-)methyltransferase. Other methyltransferases examined to date (Borchardt and Wu, 1974; Borchardt, 1975; Coward et al., 1972) are not able to tolerate to this degree such changes in the oxidation state of the sulfur atom of AdoHcy. AdoHcy sulfoxide and AdoHcy sulfone were shown earlier to have some inhibitory effect toward catechol-*O*-methyltransferase (Borchardt and Wu, 1974; Coward et al., 1972).

The lack of inhibitory activity of many of the base modified analogues of AdoHcy suggests that this methyltransferase exhibits a fairly strict specificity for the structural features of the adenine portion of AdoHcy. Two exceptions to this generality are TubHcy (7-deaza-AdoHcy) and N^6-methyl-AdoHcy. TubHcy was found to be an extremely potent inhibitor of this mRNA methyltransferase, having an inhibition constant (K_i = 0.39 ± 0.06 μM) substantially lower than AdoHcy (K_i = 1.09 ± 0.14 μM). However, it has been observed that TubHcy is a general inhibitor of all the methyltransferases examined to date (Coward et al., 1974; Borchardt et al., 1976a). Therefore, TubHcy's potential usefulness as an inhibitor of a specific transmethylation in vivo is limited, because of its general inhibitory activity and the cytotoxic effects of tubercidin, a metabolic product of TubHcy (Coward and Chang, 1975). Also of interest was the inhibitory activity of N^6-methyl-AdoHcy (K_i = 2.08 ± 0.25 μM) toward mRNA (guanine-7-)methyltransferase, since this compound has only shown significant activity toward indoleethylamine-*N*-methyltransferase (Borchardt, 1975).

From the results of these studies, it can be concluded that the Newcastle disease virion mRNA (guanine-7-)methyltransferase will tolerate changes in certain structural features of AdoHcy. For example, AdoHcy sulfoxide, AdoHcy sulfone, and N^6-methyl-AdoHcy exhibit potent and selective inhibitory activities on only this transmethylation, suggesting the possibility of designing specific inhibitors of this methyltransferase for in vivo use. It is interesting to note that RNA methylations, in general, seem to be very sensitive to inhibition by AdoHcy. For example, Glick et al. (1975) have reported the inhibition constants for AdoHcy toward three highly purified tRNA methyltransferases, each being in the range of 1–10 μM. The inhibition constant for AdoHcy toward the mRNA (guanine-7-)methyltransferase reported here was 1.09 μM. Thus the feasibility of inhibiting RNA methyltransferase in vivo is probably greater than inhibiting many small molecule methylations, which have substantially higher AdoHcy inhibition constants (Borchardt and Wu, 1974).

The results reported here and those reported earlier (Borchardt, 1976) suggest that differences do exist in the structural requirements needed for binding of AdoHcy to methyltransferases and that appropriately modified analogues of AdoHcy may be useful inhibitors of specific transmethylations. Work is presently in progress in our laboratory evaluating the inhibitory properties of these and other AdoHcy analogues on viral mRNA methylations both in vivo and in vitro.

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Vesicles of Variable Diameter Prepared by a Modified Injection Method†

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ABSTRACT: A modified injection method is described, which enables the formation of single bilayered vesicles of variable diameter (15–50 nm) in a controlled way. The vesicles produced have been characterized by light scattering, electron microscopy, and laser light scattering.

The contribution of individual membrane components to biological processes like permeation and fusion is difficult to assess from studies on "intact" membranes. Therefore it seems useful to complement these studies with measurements on simple "artificial" membranes. Model membranes such as vesicles or liposomes, prepared from components that occur naturally in biological membranes, offer possibilities of studying a system of intermediate complexity. The use of pure lipids with well-defined chain length and polar head groups is necessary if one is to understand the physical chemical behavior of these molecules in biological processes from a more fundamental point of view. However, it will first be necessary to characterize the model systems used and to find a method of obtaining these systems in a reproducible and reliable way.

Vesicles (single bilayered liposomes), which have proved useful as models for biological membranes, commonly are prepared by sonication (Huang, 1969) which entails the danger of damaging the lipid molecules (Klein, 1970; Hauser, 1971), although the damage can be minimized by carefully controlled conditions (Huang and Charlton, 1972).

An alternative way to prepare vesicles is the injection of an ethanolic solution of lipid into an aqueous (salt) solution (Batzri and Korn, 1973). This procedure has no degrading effect on the phospholipid and gives a reasonably homogeneous preparation of vesicles with a diameter of about 26.5 nm.

In this paper we show that the injection method can be used to obtain fairly monodisperse vesicles of variable size (25–120 nm) by varying the injection conditions. The presence of ethanol is a disadvantage, but this can easily be removed by dialysis. Moreover, characterization of the vesicles by light scattering shows no differences between dialyzed and nondialyzed samples.

Materials and Methods

The lecithins used were dimyristoylphosphatidylcholine (DMPC)[1] and dipalmitoylphosphatidylcholine (DPPC) obtained from Koch and Light Laboratories, Coinbrook, Bucks, England (art. 2203t, batch No. 65508 and art. 3409t, batch No. 67082, respectively). To remove a fusogenic impurity, DMPC was purified by column chromatography on a silica column (Merck, Kieselgel 60 reinst, 70–230 mesh) using a continuous gradient of chloroform and methanol (up to 70%) as eluent. Fractions were analyzed by thin-layer chromatography with a chloroform–methanol–water (65:35:4) mixture as developer. The first and last fractions showing the presence of lecithin were discarded to avoid contamination of small amounts of impurity, that would not be visible with this low sensitivity analysis. The lipid thus obtained was colorless and

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[1] Abbreviations used: DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; Tris, tris(hydroxymethyl)aminomethane.