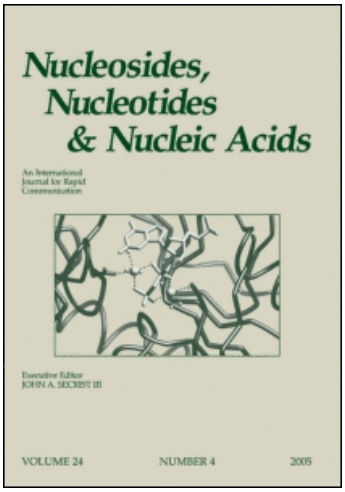

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POLYFUNCTIONAL O-SUBSTITUTED HYDROXYLAMINES: MODIFICATION OF NUCLEIC ACIDS, INHIBITION OF SAM-DECARBOXYLASE

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ABSTRACT. Treo-1-aminooxy-2,3-dihydroxy-4-mercaptobutane is suggested for the introduction of reactive thiol groups via cytidine residues and/or 3'-end of nucleic acids. S-(5'-desoxyadenosyl)-aminooxyethyl-thiomethyl hydroxylamine irreversibly inhibits SAM decarboxylase in 10^{-9} M.

Many reactions for modification of nucleic acids were suggested, but approaches for base-specific introduction of reactive groups (HS-; H_2N-; HOOC-; etc.) were not developed. Among the functions to be introduced into nucleic acids the most promising are HS- and H_2N-groups, because of high reactivity under mild conditions and sensitivity of quantative determinations. Hydroxylamine and O-methylhydroxylamine are classic reagents for RNA and DNA modification with established mechanism of reaction through cytidine residues. So, we constructed a series of polyfunctional hydroxylamines H_2N-O-R-X (X= -SH; $-NH_2$; -COOH; etc.) that reacted with tRNA like O-methylhydroxylamine.

O-methylhydroxylamine modifies DNA and RNA only at high concentrations, so hydroxylamines H_2N-O-R-X must be good soluble at neutral and slightly acidic pH (optimum is 5,0) remaining uncharged. Treo-1-aminooxy-2,3-dihydroxy-4-mercaptobutane (aminooxythiotreitol) /1/ is the simplest structure, being in accordance with the above requirements and it was used for nucleic acids modification.

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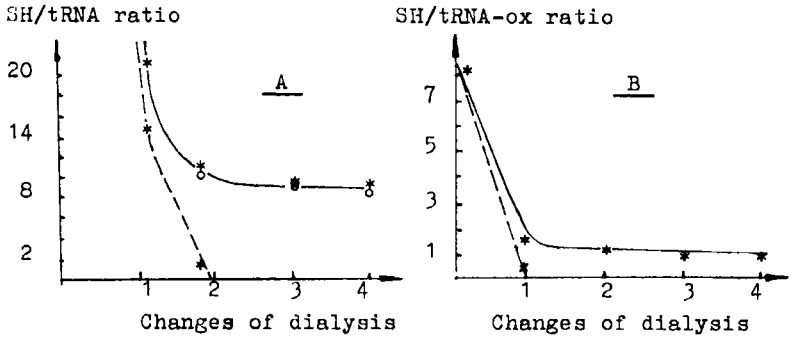

FIG. 1. Introduction of HS-groups into tRNA cytidine residues (A) and 3'-oxidised ribose (B).
A: 0,82 M aminooxythiotreitol, pH 5,0; $2,5.10^{-4}$ M tRNA (165 o.u./ml) were incubated 16 hr. at 37° (under these conditions 14-C-CH_3ONH_2:tRNA ratio is 10:1) and dialysed against 1 mM EDTA and 50 mM NaCl for 48 hr. with four buffer chainges. SH/tRNA ratio was determined with Ellman reagent (*) and o-chloromercuri-p-nitrophenol (o). In the control the same mixture was dialysed on being prepared.
B: $5,0.10^{-4}$ M tRNA-ox (330 o.u./ml) and $3,5.10^{-3}$ M 1-aminooxy-4-mercaptobutane in 0,1 M Na-acetate pH 5,0 were incubated 2 hr. at 20°. Determination of SH/tRNA ratio is described in A. In control tRNA was used instead of tRNA-ox.

Incubation of tRNA in concentrated aminooxythiotreitol solutions resulted in incorporation of eight reactive thiol groups per mole of tRNA /2/, being titrated with Ellman reagent and o-chloromercuri-p-nitrophenol (Fig. 1A)

1-aminooxy-4-aminobutane reacted with tRNA by the same way and introduced amino groups were determined by fluorescamine titration (Fig. 2).

Polyfunctional O-substituted hydroxylamines turned out to be convenient reagents for introduction of reactive groups via 3'-oxidised terminal of RNA. The morpholidate-type adducts with dilute solutions of 14-C-CH_3ONH_2 were formed rapidly and specifically trough 3'-end. We have introduced via 3'-terminal reactive: HS- and H_2N-groups (Fig. 1B and Fig. 2), as well as HOOC-; $(HO)_2(O)P$-; H_2NO-groups. ^{125}I-label and polymercurated claster were introduced by means of earlier unknown mercury-containing hydroxylamines /3/.

One of the key enzymes in polyamine biosynthesis is pyruvate-dependent decarboxylase of S-adenosylmethionine

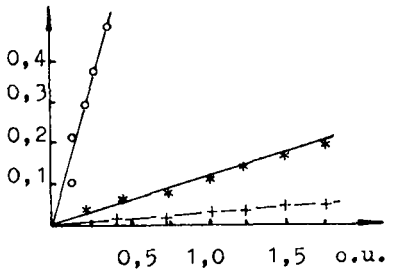

FIG. 2.

Introduction of H_2N-groups into tRNA. $1,2.10^{-4}$ M tRNA and tRNA-ox were incubated at pH 5,0 in 1,0 M and $1,2.10^{-3}$ M 1-aminooxy-4-aminobutane as described in Fig.1(A,B). After the removal of the excess of the reagent H_2N-groups introduced in cytidine residues (o) or 3'-oxydised ribose (*) were titrated with fluram (λ_{ex} 385; λ_{em} 485). (+) is the control.

TABLE 1. Irreversible inhibition of SAM decarboxylase from E.Coli and rat liver.

Compound		Preincubation 30 min (I_{50}) E.Coli	Rat liver
Ado-$\overset{+}{S}(CH_3)$-$(CH_2)_2$-ONH_2	(I)	2.10^{-8} M	3.10^{-9} M
Ado-S-$(CH_2)_2$-ONH_2	(II)	1.10^{-4} M	4.10^{-5} M
Ado-$\overset{+}{S}(CH_3)$-$(CH_2)_4$-ONH_2	(III)	9.10^{-7} M	8.10^{-9} M
Ado-S-$(CH_2)_4$-ONH_2	(IV)	5.10^{-4} M	2.10^{-4} M
H_3C-$\overset{+}{S}(CH_3)$-$(CH_2)_2$-ONH_2	(V)	2.10^{-4} M	-----
H_3C-S-$(CH_2)_2$-ONH_2	(VI)	5.10^{-4} M	-----

(SAM). Hydroxylamine-containing analogs of decarboxylated SAM were synthesised /1/ as a means for irreversible inhibition of this enzyme /4,5/.

Substance (I) was found to be 1000 times more active than the best among known inhibitors of this enzyme. The inhibition was time-dependent, substrate was uncapable to restore the enzyme activity, but it protected enzyme from (I) action. Application of Kitz-Willson's approximation for the determination of the inhibition parameters indicated on double step process. The affinity of compound (I) towards enzyme active site at the integral reversible step was 100 times higher than decarboxylated SAM had. Substance (I) interacts with the enzyme in monocation form (pK H_2NO-group is 4,5-5,0) that seems to be preferable in comparison with double cation form of decarboxylated SAM.

Specific mode of SAM-decarboxylase interaction with (I) is confurmed by low activities of (II) and (V) towards enzyme. Besides, pyridoxal-5'-phosphate dependent aspartate aminotransferase is slightly inhibited at mM concentrations of compound (I).

High potence and specificity of (I) towards SAM decarboxylase can be explained in terms of simmilarity between E-I complex and one of E-S intermediates.

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