Vol. 86, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 14, 1979 Pages 594-598

POLYAMINES INHIBIT DNA METHYLATION IN VITRO

Ray Cox

Veterans Administration Hospital
Department of Biochemistry
University of Tennessee
Center for the Health Sciences
Memphis, TN 38104 (U.S.A.)

Received November 4, 1978

SUMMARY

DNA methylase from rat liver differs from RNA methylase in response to polyamines, being inhibited rather than stimulated. With methyl-deficient DNA from rat liver as substrate, the DNA methylase is inhibited 97%, 59% and 42% respectively, by spermine, spermidine and putrescine at 1 mM concentration.

INTRODUCTION

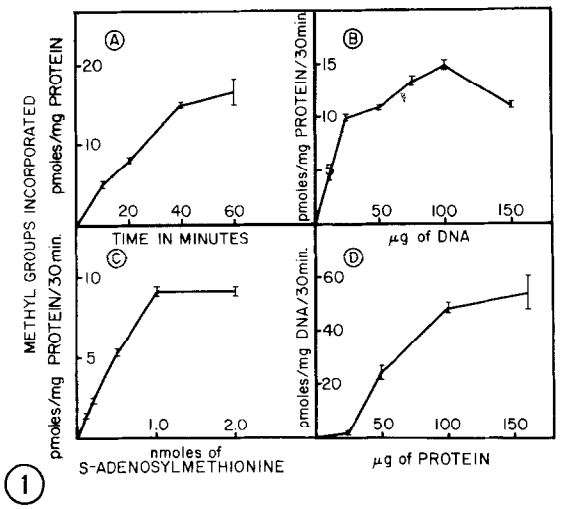
Polyamines bind to nucleic acids (1,2), and possibly a structural alteration in the RNA molecule occurs. They also stimulate RNA methylation in vitro (3-5). The recent purification of DNA methylase (6-8) and availability of methyl-deficient DNA from rat liver (9) has allowed us to examine the homologous system and to observe the effects of polyamines. The results are reported in this study.

MATERIALS AND METHODS

S-Adenosyl-[Me-^{3}H]-methionine with a specific activity of 9.7 Ci/mmol was obtained from New England Nuclear Corp., Boston, MA. S-Adenosylmethionine iodide, putrescine, spermidine and spermine were purchased from Sigma Biochemical Corp., St. Louis, MO. Male Sprague Dawley rats weighing 110-160 g obtained from the Charles River Breeding Lab., Inc., Wilmington, MA were used as a source of rat liver DNA and DNA methylase.

DNA Methylase Preparation: The methylase was solubilized according to Roy and Weissbach (6). Rat liver nuclei were isolated as previously described (10) and resuspended in 1 mM EDTA, 0.5 mM dithiothreital, 10% glycerol, and 50 mM Tris HCl, pH 7.8, at 15 mg of nuclear protein per ml. Protein was determined by the Lowry method (11). DNA methylase was solubilized by adding 4 M NaCl to a final concentration of 0.25 M NaCl and the resuspension centrifuged at 10,000 x g for 30 minutes. The pellet containing mostly DNA was discarded, DEAE cellulose was added to the supernatant to give a 30% slurry and the mixture shaken in the cold for 30 minutes and DEAE cellulose removed by a 10 minute centrifugation at 3°C and 10,000 x g. For complete removal of the DEAE cellulose, the supernatant was centrifuged again and used as DNA methylase.

0006-291X/79/030594-05$01.00/0



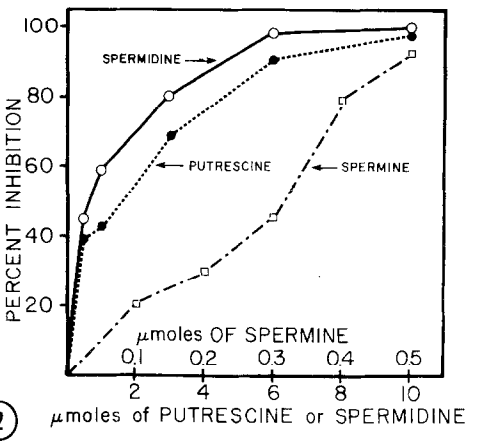


Fig. 1. Properties of DNA Methylase. A - Time curve. The reaction was run at varying times with 25 μg of methyl-deficient DNA and 100 μg of protein from the partially purified enzyme. B - DNA curve. Varying amounts of DNA were incubated with 100 μg of protein for 30 minutes. C - S-Adenosylmethionine curve. The reaction was run for 30 minutes with 100 μg of protein, 25 μg of DNA, and varying amounts of S-adenosylmethionine. D - Protein curve. Varying amounts of protein were incubated for 30 minutes with 25 μg of DNA. Each point is the mean of triplicate assay tubes. Bar, S. E. See Materials and Methods for other assay conditions and work-up of the assay.

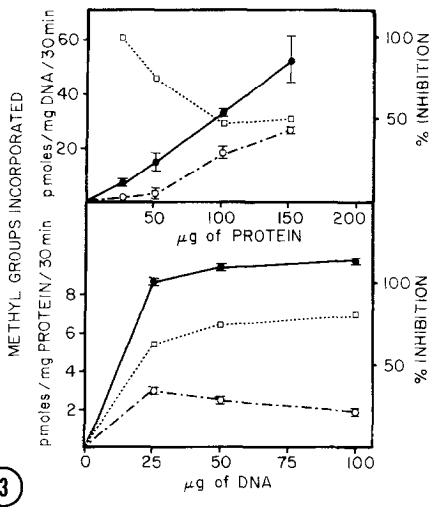
Fig. 2. Inhibition of DNA methylase by polyamines. Varying amounts of the polyamines were added to the assay. The reaction was run for 30 minutes with 100 μg of protein, 25 μg of DNA, and 1 nanomole of S-adenosylmethionine, which gave a control value of 9.1 ± 0.8 picomoles of methyl groups incorporated per mg of protein. Each point was determined from the mean of triplicate assay tubes. □----□ - spermine, o——o - spermidine, ●----● - putrescine.

Methylation Assay: The DNA methylase was incubated with S-adenosyl-[Me-^{3}H]-methionine (2.87 μM, 2.5 μCi/nmol) in a total volume of 350 μl containing 10 mM EDTA and 50 mM Tris-HCl at pH 7.4. Methyl-deficient DNA was prepared from rat liver as previously described (9) and used as the substrate. The mixture was incubated for 30 minutes at 37°C in a shaking water bath and the reaction stopped by adding 0.3 ml of 2 M NaCl and chilling in ice. RNA and protein were removed as previously described (12) and the DNA hydrolyzed in 1 ml of 0.6 N perchloric acid at 70° for 10 minutes. Aliquots were counted for radioactivity and DNA was determined by the diphenylamine reaction (13). The radioactivity was counted in 10 ml of scintillation fluid containing toluene, Triton X-100, and PPO with a Packard liquid scintillation counter.

5-Methylcytosine was identified as the product of the reaction by enzymatic and acid hydrolysis of the purified DNA with column and paper chromatography as previously described (12).

RESULTS

Fig. 1 shows that the methyl incorporation into DNA was dependent upon the time of incubation, the concentration of both substrates, S-adenosylmethionine and DNA, and the presence of enzyme. It is interesting to note in Fig. 1 at low protein concentrations (below



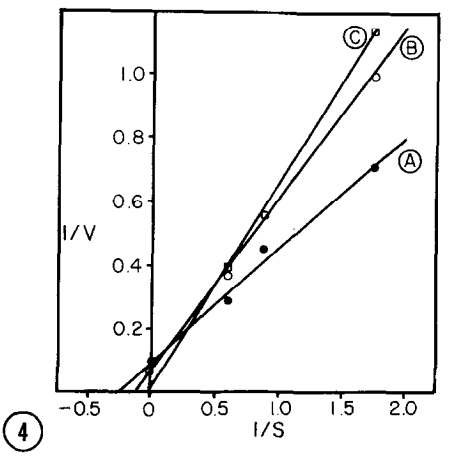


Fig. 3. Effect of protein and DNA on the inhibition of DNA methylase. The reaction was run for 30 minutes with 1 nanomole of S-adenosylmethionine and varying amounts of protein or DNA in the presence of 1 mM spermidine. □----□ - % inhibition, ●——● - no inhibitor, o----o - with 1 mM spermidine.

Fig. 4. Lineweaver-Burk plot of substrate and inhibition by spermine. The reaction was run for 30 minutes with 100 μg of protein, 25 μg of DNA and varying concentrations of S-adenosylmethionine. A - no inhibitor, B - 0.1 mM spermine, C - 0.2 mM spermine. The y intercept values for A, B, and C are 0.11, 0.08, and 0.005, respectively. Correlation coefficients for curves A, B, and C were 0.9919, 0.9988, and 0.9995 respectively.

25 μg), there was no methylation of DNA. Thus, some unknown co-factor may be required for this reaction to occur; and as the protein concentration is increased, the amount of this co-factor is also increased allowing the reaction to proceed. Removal of EDTA from the standard assay inhibited the reaction by 73%. This may be a reflection of the endogenous level of Mg^{++}, since the addition of 50 mM Mg^{++} inhibited the reaction by 91%.

The diamine, putrescine, and the polyamines, spermidine and spermine were added to the methylation assay as described in Fig. 2. DNA methylation was inhibited by each of the amines, and at 1 millimolar, spermine, spermidine and putrescine inhibited the methylase reaction 97%, 59% and 42% respectively.

Fig. 3 shows the effect of protein or DNA on the inhibition of DNA methylation at a constant amount of spermidine. The percent inhibition shows a small increase with the varying amounts of DNA. However, with protein, the percent inhibition is decreased with increasing protein.

Substrate (S-adenosylmethionine) concentration curves were run in the presence and absence of each amine at a concentration that yields 25% to 50% inhibition. At two different concentrations of inhibitor, a mixed type of inhibition was observed. Fig. 4 shows the Lineweaver-Burk plots obtained with spermine, which is typical of all 3 amines. From these data, we are unable to suggest a specific type of inhibition.

DISCUSSION

The inhibition of DNA methylation by these polyamines is somewhat surprising, since RNA methylation in vitro is stimulated by the addition of millimolar concentrations of polyamines (3-5). The data in Fig. 3 suggests that the polyamines are acting on the methylase enzyme, since an addition of the methylase extract decreased the inhibition. Another possible explanation for this observed effect of the enzyme extract is that RNA, present in the enzyme extract, is titrating out the polyamine. We have not determined the RNA content of the enzyme preparation. Fig. 4 suggests that the inhibition is a mixed type. However, at the lower inhibitor concentration, the y intercept value 0.07 is closer to y intercept value 0.1 with no inhibitor than the value 0.004 at the higher inhibitor concentration. This suggests that at lower inhibitor concentrations the inhibitor first binds to the enzyme, perhaps in a competitive fashion. Since it is known that polyamines bind to nucleic acids at the higher concentration of inhibitor, the binding to DNA might alter its structure in such a way that methylation is inhibited. Thus, the polyamines may affect both the enzyme and DNA, producing a mixed type of inhibition.

The propylamine group of the polyamines is derived from S-adenosylmethionine, which is the substrate for DNA methylase. Thus, the polyamines might bind through its propylamine group to the active site of the enzyme and at the same time with other amine groups of the same molecule bind to the negative charges of the DNA. This may produce the mixed type of inhibition observed. The inhibition of DNA methylation by polyamines in vitro, could merely be a non-specific type of binding between the positive charges of the polyamines and the negative charges of the DNA, and have no biological significance. Since the polyamines are found in the nucleus associated with DNA, and since spermine is a good inhibitor of this reaction in vitro, it is interesting to postulate a biological role for

the polyamines in DNA methylation. The polyamines may serve as a safety valve for preventing the hyper methylation of DNA. For example, when S-adenosylmethionine is increased, overt methylation of DNA might occur. To prevent this, the cell shunts the S-adenosylmethionine to polyamine synthesis, which lowers the level of S-adenosylmethionine and increases the concentration of polyamines. The increased polyamines then inhibit the methylation of DNA. Thus, by decreasing the S-adenosylmethionine levels and inhibiting DNA methylation at the same time, the cell regulates the amount of methyl groups incorporated into DNA.

ACKNOWLEDGEMENTS

The author is grateful for the expert technical assistance of Charles Prescott and Karen Seay. This work was supported by the U. S. Veterans Administration (4323-01) and by USPHS Research Grant CA-15189 from the National Cancer Institute.

REFERENCES

1. Bachrach, U. (1973) Function of Naturally Occurring Polyamines, pp. 63-73, Academic Press, New York.
2. Cohen, S. S. (1977) in Advances in Polyamine Research, Vol. I, pp. 110, Raven Press, New York.
3. Leboy, P. S. (1970) Biochemistry 9, 1577-1584.
4. Pegg, A. E. (1971) Biochim. Biophys. Acta 232, 630-642.
5. Young, D. V. and Srinivasan, P. R. (1971) Biochim. Biophys. Acta 238, 447-463.
6. Roy, P. H. and Weissbach, A. (1975) Nucleic Acids Res. 2, 1669-1684.
7. Sneider, T. W., Teague, W. M., and Rogachevsky, L. M. (1975) Nucleic Acids Res. 2, 1685-1700.
8. Turnbull, J. F. and Adams, R. L. P. (1976) Nucleic Acids Res. 3, 677-695.
9. Cox, R. and Irving, C. C. (1977) Cancer Res. 37, 222-225.
10. Cox, R., Martin, J. T., and Shinozuka, H. (1973) Lab. Invest. 29, 54-59.
11. Lowry, O. H., Rosebrough, N. J., Fan, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
12. Cox, R., Prescott, C., and Irving, C. C. (1977) Biochim. Biophys. Acta 474, 493-499.
13. Burton, K. (1956) Biochem. J. 62, 315-323.