Amino Acids (2007) 33: 267–272
DOI 10.1007/s00726-007-0535-y
Printed in The Netherlands

Amino Acids

Antiviral activity of oxidized polyamines

Review Article

U. Bachrach

Department of Molecular Biology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Received October 31, 2006
Accepted February 1, 2007
Published online April 12, 2007;

Summary. Polyamines, oxidized by serum amine oxidase, yield aminoaldehydes and hydrogen peroxide. Acrolein may be formed from the aminoaldehydes by a spontaneous β-elimination process. These oxidation products "oxidized polyamines" inhibit bacterial growth and exhibit anticancer activity. The antimicrobial activity of oxidized polyamines is not limited to bacteria; and the inactivation of bacterial viruses, plant viruses and animal viruses, was also reported. Bacteriophages of the T-odd series are permeable and were inactivated by oxidized polyamines. The inactive phages absorb to their bacterial host and injected their DNA, which formed a stable inactive complex with the aminoaldehydes. Aminoaldehydes, synthesized chemically, also inactivated viruses. The growth of the plant viruses: Tobacco mosaic virus, Potato virus X and Alfalfa mosaic virus was also inhibited by oxidized polyamines. The animal viruses, which were inactivated by oxidized polyamines included Myxoviruses (influenza and Newcastle disease viruses), West Nile, vaccinia and Sindbis viruses. These findings may have practical implications.

Keywords: Oxidized polyamines – Aminoaldehydes – Acrolein – Inactivation of bacteriophages – Plant viruses – Animal viruses

Abbreviations: Ox. Sp., Oxidized spermine; p.f.u., plaque forming units; SAO, serum amine oxidase

Introduction

The polyamines spermine, spermidine and the diamine putrescine are important cellular effectors, playing key roles in DNA, RNA and membrane stabilization, DNA replication, transcription, protein synthesis and ion channel modulation (Cohen, 1998; Childs et al., 2003; Wallace et al., 2003). The intracellular concentrations of polyamines can be regulated by uptake, synthesis, excretion, modification (acetylation) and oxidation.

Polyamines are oxidized by serum amine oxidase (SAO); yielding aminoaldehydes and hydrogen peroxide (Tabor et al., 1964). Under certain conditions, the labile aminoaldehydes are spontaneously converted into acrolein by a β-elimination process (Alarcon, 1964).

The oxidation products of polyamines, such as oxidized spermine (Ox. Sp.), are highly cytotoxic. They are anticancer (Bachrach et al., 1967; Averill-Bates et al., 1994; Agostinelli et al., 2004; Toninello et al., 2006), inhibit bacterial growth (Bachrach and Persky, 1964), are antihelminthic (Ferrante et al., 1986) and prevent the multiplication of malaria (Rzepczyk et al., 1984; Morgan et al., 1986) and trypanosome parasites (Ferrante et al., 1984).

Bacterial viruses–bacteriophages

Various antibacterial and anticancer drugs are known, but our arsenal to combat viral diseases is rather limited. It was therefore of interest to find out whether oxidized polyamines also inhibit the growth of viruses, and if they do, what is the mechanism of viral inactivation.

Bacterial viruses (bacteriophages) infect bacteria and provide a very convenient system to study antiviral activities. The bacterial host can be grown easily and progeny phages appear in the culture, 20–30 min after infection. Moreover, the mechanism of infection, phage genetics and the molecular events related to phage multiplications have been studied extensively. *Escherichia coli* is the most common host, which can be infected by the so called coliphages. They can be divided into double-stranded DNA phages (mainly of the T series), single stranded DNA phages (such as $\phi \times 174$) and RNA phages (such as MS2). It is therefore not surprising that phage-bacteria systems served as an early model to study the antiviral

Table 1. Effect of oxidized spermine on bacteriophages

Plaque forming units (p.f.u.)				
Phage	Control	Ox. Sp.	Inactivation ($\log_{10}$)	
T1	1.8×10^9	1.2×10^3	6	Permeable
T3	1.1×10^8	9.1×10^4	4	Permeable
T5	2.3×10^8	1.1×10^5	3	Permeable
T7	9.6×10^8	1.5×10^3	5	Permeable
T2	1.0×10^8	1.7×10^8	0	Non-permeable
T4	1.7×10^8	1.1×10^8	0	Non-permeable
MS2	8.0×10^8	1.0×10^4	4	RNA phage

Bacteriophages were incubated for 90 min at 37 °C with 1.5 μmol/ml of oxidized spermine and viable phages were counted
Ox. Sp. Oxidized spermine

activity of oxidized polyamines (Bachrach et al., 1963; Nishimura et al., 1971, 1972). It soon became apparent that bacteriophages differ in their susceptibility to Ox. Sp. Coliphages of the T-odd series were sensitive, while coliphages of the T-even series were resistant and were not inactivated by Ox. Sp. (Bachrach, 1970). The titer of RNA phages was also reduced significantly after exposing the viruses to Ox. Sp. (Table 1). The selective susceptibility of the T-even and T-odd series to Ox. Sp. can be explained by the well known fact that T-odd coliphages are permeable, unlike coliphages of the T-even series. This would imply that Ox. Sp. has to penetrate into the phage (head?) to exert cytotoxicity.

The following are the different steps in phage infection and propagation:

- Phage adsorption to the bacterial host
- Injection of phage DNA (or RNA) into the bacterial host
- Phage maturation (DNA replication and protein synthesis)
- Disruption of the bacterial host and release of progeny phages

Phage adsorption

Electron microscopy clearly showed that inactive T5 coliphages adsorb to their host (Bachrach, 1970). The ability of inactivated bacteriophages to absorb to their host was further confirmed by using radioactive phages. To find out whether inactivated T5 bacteriophages, inject their DNA into the bacterial host, the classical Hershey and Chase (1952) method was used. Phage DNA and proteins were labeled with P^{32} and S^{35}, respectively. Radioactive bacteriophages were incubated with their bacterial host, and free phages were removed by centrifugation. Thereafter, the sediment, containing bacteria and adsorbed coliphages, was subjected to shearing in a Waring blendor. After another centrifugation, the supernatant fluid contained intact bacteriophages (containing DNA) or empty ''ghosts'', which are phage envelopes devoid of injected DNA. On the other hand, the sediment contained infected bacteria, into which phage DNA was injected. Table 2 clearly shows that radioactive phages were adsorbed to their bacterial host and that P^{32}-labeled DNA was injected.

Table 2. Injection of DNA from inactivated phages

Phage	Labeling	Blendor mixture (cpm)	DNA injected (cpm)	%
T5 + Ox. Sp.	P^{32}	76,320	32,000	58
T5	P^{32}	114,380	52,400	53
+Ox. Sp.	H^3 Ox. Sp.	30,080	14,520	49

Bacteriophages were incubated for 90 min at 37 °C with 1.5 μmol/ml of oxidized spermine and viable phages were counted
Ox. Sp. Oxidized spermine

Injection phage DNA

Aliquots of radioactive T5 bacteriophages were treated with H^3-labeled Ox. Sp. for 90 min and excess of the cytotoxic agent was removed by dialysis. It may be seen (Table 2) that the P^{32}-labeled DNA was injected into the host, as was the DNA of oxidized spermine-inactivated phages. Table 2 also shows that radioactive oxidized spermine was transferred into the bacterial host along with the injected DNA.

It may therefore be concluded that:

1. To inactivate bacteriophages, Ox. Sp. had to penetrate into phage head.
2. Oxidized spermine did not prevent the adsorption of the inactivated bacteriophages to the bacterial host.
3. DNA from the inactivated bacteriophages was injected into the bacterial host.
4. Oxidized spermine, formed a complex with bacteriophage-DNA, and this (inactive?) complex was injected into the bacterial host.

Plant viruses

After finding that permeable coliphages can be inactivated by oxidized polyamines, plant viruses were examined. These included: Potato virus X (PVX), Tobacco mosaic virus (TMV), Alfalfa mosaic virus (AMV). When TMV viruses were treated with Ox. Sp. (100 μg/ml) for

180 min and then rubbed on tobacco leaves, only 5% of the control lesions were detected (Bachrach et al., 1965). Oxidized polyamines had no deleterious effect on plant leaves. Similar results were obtained with the other plant viruses.

Animal viruses

Obviously, the inactivation of animal viruses appeared to be most interesting. Many of these viruses cause human and animal diseases and the number of antiviral agents, used for therapy, is rather limited. When suspensions of Newcastle disease viruses (which infect poultry), were incubated for 3 h with Ox. Sp. (100 μg/ml), the number of plaque forming units (p.f.u.) was reduced significantly (Table 3). Other viruses, such as influenza virus and West Nile viruses, which infect human beings, were also inactivated (Katz et al., 1967). It is evident from Table 3 that the titer of West Nile and Sindbis viruses was reduced by more than 4 logs. The rate of inactivation of Influenza and Newcastle disease viruses was more moderate (Table 3). A better inactivation could be achieved by increasing the dose of Ox. Sp. and the time of drug exposure. The effect of incubation time on the inactivation of Newcastle disease viruses is summarized in Table 4. It is clear that the rate of inactivation was time-dependent and a reduction of 5.5 $\log_{10}$ was observed after exposure to enzymatically-prepared Ox. Sp. for 5 h. Aminoaldehydes, resembling Ox. Sp. can be prepared chemically (Bachrach and Rosenkranz, 1969). As these compounds can be obtained in a pure and stable form, the effects of hydrogen peroxide and acrolein can be ruled out. Interestingly, the synthetic aminoaldehyde was more active compared with the enzymatically prepared Ox. Sp. and a complete inactivation of the viruses was observed after exposure for 5 h (Table 4). The chemical synthesis of aminoaldehydes permitted the synthesis of analogs. In one of these synthetic products, the diamino-butane central moiety of Ox. Sp. was replaced by diamine-hexane (Diamino-hexane in Table 4). This analog was more active when compared to the enzymatially prepared Ox. Sp. (Table 4).

Table 3. Inactivation of animal viruses by oxidized spermine

Virus	Inactivation ($\log_{10}$)
Influenza virus	2.7
Newcastle disease	1.9
West Nile	4.4
Sindbis	4.3

Oxidized spermine 100 μg/ml was incubated with viruses at 37 °C for 3 h

Table 4. Inactivation of Newcastle disease virus by amino aldehydes

Time (h)	Compound (0.8 mM)	p.f.u./ml	Inactivation ($\log_{10}$)
0	–	8.7×10^7	–
1	Ox. Sp. (enzymatic)	8.2×10^6	1.0
3	Ox. Sp. (enzymatic)	4.4×10^4	3.3
5	Ox. Sp. (enzymatic)	2.5×10^2	5.5
1	Ox. Sp. (synthetic)	4.7×10^4	3.3
3	Ox. Sp. (synthetic)	10	6.0
5	Ox. Sp. (synthetic)	0	7.0
1	Diamino-hexane	1.2×10^4	3.8
3	Diamino-hexane	10	6.0

Oxidized polyamines were incubated with viruses at 37 °C for 3 h
p.f.u. Plaque forming units
Diamino-hexane-synthetic aminoaldehydes with a diamino-hexane backbone

Enzymatically-prepared Ox. Sp. and the synthetic analogs, did not bind to viral envelopes and the process of infection was not impaired. An indication for the intactness of the inactivated Newcastle disease virus, was obtained by cellular fusion experiments. Some members of the Myxovirus family, posses the ability to fuse certain animal cells to form giant polykaryocytes. Viruses totally inactivated by formaldehyde lost most of their fusion ability, as the viral envelope proteins were partially denatured. Thus, the fusion ability of totally oxidized spermine-inactivated viruses, would suggest that the viral envelope remained intact. The fusion experiment was carried out by incubating Ehrlich ascites cells with live or inactivated Newcastle disease viruses. After 1 h the cells were examined under a phase microscope. These experiments clearly showed that the inactivated viruses were able to fuse the Ehrlich ascites cells and giant polykaryocytes were formed (Bachrach, 1970). It can therefore be concluded that viruses inactivated by Ox. Sp. adsorb to their host and that the viral envelope remained intact.

Another evidence for the integrity of the envelopes of the inactivated viruses was provided by hemagglutination studies (Kremzner and Harter, 1970). Influenza and Newcastle disease viruses have the ability to hemagglutinate chick red blood cells. This reflects the intactness of their viral receptor sites. Indeed, when either influenza or Newcastle disease viruses were incubated for 24 h with oxidized spermine or spermidine (1.65 μmol/ml) and then treated with chick red blood cells, the inactivated Myxoviruses retained the integrity of the viral hemagglutinins and the titer of hemagglutination was higher than that observed when formalin-inactivated viruses were used.

It may therefore be concluded that oxidized polyamines are not bound to viral envelopes and most likely penetrate into viral particles and interact with their nucleic acids (Kremzner and Harter, 1970; Bachrach and Don, 1971).

Experiments were carried out to find out whether Ox. Sp. forms a complex with the RNA of the inactivated viral particle. Oxidized spermine was labeled with C^{14} and the RNA of influenza viruses contained H^3-labeled uridine. If Ox. Sp. was preferentially bound to the RNA of the virus, then the ratio of radioactive C^{14}/H^3 for the intact oxidized spermine-treated virus particles should resemble that of phenol-extracted viral RNA. On the other hand, if most of the Ox. Sp. is bound to the viral envelope, then a different C^{14}/H^3 ratio would be obtained for the intact viruses and their RNA. When H^3-labeled influenza viruses were treated with C^{14}–Ox. Sp. (1.4 μmol/ml), the ratio of C^{14}/H^3 was 0.83 for the intact viral particles, and that of the phenol-extracted RNA was 0.82. This observation for influenza viruses resembles that observed with coliphages; therefore a similar mode of inactivation can be envisaged.

To ascertain that the carbonyl groups of oxidized polyamines were essential for the antiviral activity, sodium borohydride was used. This compound, which reduces the aldehydes to the corresponding alcohols, was incubated at 20 °C for 10 min with oxidized polyamines (1.65 μmol/ml). Influenza viruses retained their viability after incubation with the reduced compound for 24 h (Bachrach and Don, 1971). These experiments clearly showed that carbonyl groups were essential for viral inactivation and that the reduced derivative lacked antiviral activity.

Discussion

Several factors generated from polyamines during oxidation by SAO, have been considered as inhibitors of cell growth. These include: amino-aldehydes, hydrogen peroxide and ammonia. In addition, the labile amino-aldehydes may undergo a spontaneous β-elimination to produce acrolein. It was not clear which of these products are cytotoxic.

Hydrogen peroxide

According to Sharmin et al. (2001), hydrogen peroxide was not the major cytotoxic product of polyamine oxidation and catalase did not reverse the inhibition of growth of cultured cells. Similar results were obtained by Rzepeczyk et al. (1984), who studied the effect of Ox. Sp. on Plasmodium falciparum. They found that catalase did not protect the parasites. On the other hand, Averill-Bates et al. (1994) demonstrated that catalase completely inhibited the toxic effect of oxidized polyamines when tested against cultured ovary hamster cells. They concluded that both hydrogen peroxide and aldehydes contribute to the toxicity of oxidation products of spermine. Klebanoff and Kazazi (1995) studied the inactivation of human immunodeficiency virus by spermine in the presence of SAO. They suggested that hydrogen peroxide was the active oxidation product. They speculated that peroxidase protected HIV viruses during the oxidation of spermine in semen and vaginal fluids.

Acrolein

Sharmin et al. (2001) claimed that acrolein is a major toxic compound, produced during the oxidation of polyamines by SAO. This product appeared to inhibit the growth of various cultured cells including NIH_3T_3 fibroblasts. The inhibition was prevented by aldehydes dehydrogenase. Ferrante et al. (1984), who studied the effect of Ox. Sp. on Trypanosoma lewisi, suggested that acrolein did not participate in the killing of the parasites. They measured the amount of acrolein in the oxidized spermine-treated cells and found that it was below the minimal toxic level (only 1:10-th of the initial spermine concentration was converted into acrolein). They postulated that the primary oxidation products, namely the amino-aldehydes, react with the parasites soon after their formation and only a limited part will undergo β-elimination. When the helminth Schistosoma mansoni was exposed to spermine and SAO, only a small amount of acrolein was formed. More than 5 times of acrolein was required to get the same effect level of damage as expected to be generated (Ferrante et al., 1986). Kremzner and Harter (1970), who studied the effect of Ox. Sp. on vesicular stomatitis virus, also concluded that acrolein was not the major toxic agent. Similar results were obtained by Bachrach and Rosenkovitch (1972), who studied the effect of Ox. Sp. on vaccinia virus. Nishimura et al. (1971) studied the infection of *E. coli* spheroplasts by the single-stranded $\phi \times 174$ phage and concluded that acrolein was not the active component of Ox. Sp. Nishimura et al. (1972) also reported that the phagocidal action of Ox. Sp. on T5 phages was 1000 times greater than that of acrolein. Oxidized spermine did not inactivate coliphages of the T-even series, while acrolein was toxic (Bachrach et al., 1971). It therefore appears that acrolein is not the major growth inhibitor, produced during the oxidation of polyamines by SAO.

Aminoaldehydes

There is a disagreement whether hydrogen peroxide or acrolein are the main toxic oxidation product formed after oxidizing polyamines by SAO. It is however clear that aminoaldehydes contribute much to the toxicity of oxidized spermine.

This was deduced by the following experiments:

1. Ion exchange chromatography permits the separation between the charged amino-aldehydes and acrolein or hydrogen peroxide. Aminoaldehydes, thus prepared showed significant antiparasitic activity (Morgan et al., 1986).
2. Gel filtration was also used to remove proteins and to eliminate hydrogen peroxide (Bachrach, 1970). ''Oxidized spermine'', thus prepared showed inhibition of growth of coliphages (Table 1).
3. Kremzner and Harter (1970) used phosphorylated cellulose columns to purify aminoaldehydes, formed during the oxidation of spermine by SAO. They found, that the purified oxidation product inhibited the growth of influenza and polio viruses.
4. Synthetic amino-aldehydes provided the best evidence that these oxidation products are cytotoxic. Moreover, the synthetic method permitted the formation of analogs, which proved to be more active than the enzymatically prepared Ox. Sp. (Table 4). It is to be expected that the synthetic amino-aldehydes react with the viruses soon after their exposure, so that the formation of acrolein will be minimal.

Oxidized polyamines inhibit the growth of plant viruses. These findings may be used to obtain better crops and germ free seeds.

The inactivation of various animal viruses by oxidized polyamines (Tables 3, 4) is most interesting. If our hypothesis is correct, and that oxidized polyamines are not bound to the viral envelope, but they cross the membrane and interact with viral nucleic acids, then new approaches to cure viral diseases may be envisaged. Agostinelli et al. (2004) already pointed out that polyamine-amine-oxidase systems may offer great opportunities to develop novel classes of therapeutic agents. It should also be remembered that oxidized polyamines are naturally occurring oxidation products- this may prevent criticism for medical application.

Acknowledgement

The important contribution of S. Don and R. Damboritz is greatly appreciated.

References

Agostinelli E, Arancia G, Vedova LD, Belli F, Marra M, Salvi M, Toninello A (2004) The biological function of polyamine oxidation products by amine oxidases: perspectives of clinical applications. Amino Acids 27: 347–358

Alarcon RA (1964) Isolation of acrolein from incubated mixtures of spermine with calf serum and its effect on mammalian cells. Arch Biochem Biophys 106: 240–242

Averill-Bates DA, Agostinelli E, Przybytkowski E, Mondovi B (1994) Aldehyde dehydrogenase and cytotoxicity of purified bovine serum amine oxidase and spermine in Chinese hamster ovary cells. Biochem Cell Biol 72: 36–42

Bachrach U (1970) Oxidized polyamines. Ann New York Acad Sci 171: 939–956

Bachrach U, Abzug S, Bekierkunst A (1967) Cytotoxic effect of oxidized spermine on Ehrlich ascites cells. Biochim Biophys Acta 134: 174–181

Bachrach U, Don S (1971) Inactivation of Myxoviruses by oxidized polyamines. J Gen Virol 11: 1–9

Bachrach U, Don S, Wiener H (1971) Antivirus action of acrolein, glutaraldehyde and oxidized spermine. J Gen Virol 13: 415–422

Bachrach U, Persky S (1964) Antibacterial action of oxidized spermine. J Gen Microbiol 37: 195–204

Bachrach U, Rabina S, Loebenstein G, Eilon G (1965) Antiviral action of oxidized spermine-inactivation of plant viruses. Nature 208: 1095–1096

Bachrach U, Rosenkovitch E (1972) Effect of oxidized spermine and other aldehydes on the infectivity of vaccinia virus. Appl Microbiol 23: 232–235

Bachrach U, Rosenkranz HS (1969) Inhibition of bacterial macromolecular synthesis by the polyamine POX-3. Biochem Pharmacol 18: 243–246

Bachrach U, Tabor CW, Tabor H (1963) Inactivation of bacteriophages by oxidized spermine. Biochim Biophys Acta 78: 768–770

Childs AC, Mehta DJ, Gerner EW (2003) Polyamine-dependent gene expression. Cell Mol Life Sci 60: 1394–1406

Cohen SS (1998) A guide to the polyamines. Oxford University Press, New York

Ferrante A, Ljungstrom I, Rzepczyk CM, Morgan DM (1986) Differences in sensitivity of Schistosoma mansoni schistosomula, Dirofilaria immitis microfilariae, and Nematospiroides dubius third-stage larvae to damage by the polyamine oxidase-polyamine system. Infect Immun 53: 606–610

Ferrante A, Rzepczyk CM, Saul A (1984) Polyamine oxidase-mediated trypanosome killing: the role of hydrogen peroxide and aldehydes. J Immunol 133: 2157–2162

Hershey AD, Chase M (1952) Independent functions of viral protein and nucleic acid in growth of bacteriophage. J Gen Physiol 36: 39–56

Katz E, Goldblum T, Bachrach U, Goldblum N (1967) Antiviral action of oxidized spermine: inactivation of certain animal viruses. Israel J Med Sci 3: 575–577

Klebanoff SJ, Kazazi F (1995) Inactivation of human immunodeficiency virus type 1 by the amine oxidase-peroxidase system. J Clin Microbiol 33: 2054–2057

Kremzner LT, Harter DH (1970) Antiviral activity of oxidized polyamines and aldehydes. Biochem Pharmacol 19: 2541–2550

Morgan DM, Bachrach U, Assaraf YG, Harari E, Golenser J (1986) The effect of purified aminoaldehydes produced by polyamine oxidation on the development in vitro of Plasmodium falciparum in normal and glucose-6-phosphate-dehydrogenase-deficient erythrocytes. Biochem J 236: 97–101

Nishimura K, Komano T, Yamada H (1971) Phagocidal effects of acrolein. Biochim Biophys Acta 247: 153–156

Nishimura K, Komano T, Yamada H (1972) Effects of oxidized spermine and acrolein on the transforming activity of T4 DNA. Biochim Biophys Acta 262: 24–31

Rzepczyk CM, Saul AJ, Ferrante A (1984) Polyamine oxidase-mediated intraerythrocytic killing of Plasmodium falciparum: evidence against the role of reactive oxygen metabolites. Infect Immun 43: 238–244

Sharmin S, Sakata K, Kashiwagi K, Ueda S, Iwasaki S, Shirahata A, Igarashi K (2001) Polyamine cytotoxicity in the presence of bovine serum amine oxidase. Biochem Biophys Res Commun 282: 228–235

Tabor CW, Tabor H, Bachrach U (1964) Identification of aminoaldehydes produced by the oxidation of spermine and spermidine with purified plasma amine oxidase. J Biol Chem 239: 2194–2203

Toninello A, Pietrangeli P, De Marchi U, Salvi M, Mondovi B (2006) Amine oxidase in apoptosis and cancer. Biochim Biophys Acta 1765: 1–13

Wallace HM, Fraser AV, Hughes A (2003) A perspective in polyamine research. Biochem J 376: 1–14

Author's address: Dr. Uriel Bachrach, Department of Molecular Biology, Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel, Fax: +972-2-6784010, E-mail: bachur@md.huji.ac.il