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Oxidative deamination of new polyamine analogs by bovine serum amine oxidase: a kinetic study

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Natural polyamines putrescine, spermidine and spermine are ubiquitous polycationic compounds present in significant amounts in nearly every prokaryotic and eukaryotic cell type. Spermidine and spermine primarily exist in aqueous solution at pH 7.4 as fully protonated polycations (1). Such ubiquitous chemical entities play an important role in cell growth and proliferation, in the synthesis of proteins and nucleic acids, in both normal and cancer cells.

The low efficacy of drugs currently used in anticancer therapeutic applications and the development of multidrug-resistance prompted us to study the polyamine pathway as a possible target for the development of new anti-proliferative agents.

In order to develop new amino oxidase (AO) spermine-based ligands with the aim to improve their deamination kinetic parameters, we designed and synthesized several spermine analogs, starting from its scaffold by inserting different substituents, endowed with diverse chemical and physical properties (Table 1). The future perspectives of these compounds is to be used alone or in combination with AO, purified from bovine serum, to observe their cytotoxic effect on human tumor cell lines. Among its numerous functions, the physiological role of bovine serum amine oxidase (BSAO) have been recognized to take part in protein post-translation modification. Moreover, BSAO behaves as a free radical scavenger, and protects the isolated heart against dangerous effects of the reactive oxygen intermediates.

A kinetic study was performed on several new polyamine analogs with the aim to obtain molecules that show kinetic parameters K_m and k_{cat} (k_c) better than those of the physiological polyamines, like spermine and spermidine. The study was carried out in the presence of BSAO, in buffer phosphate (BP) at physiological pH 7.4 and in several experimental conditions, using polyamine analogs in the range between 0.05-1 mM. In this system BSAO catalyzes the oxidative deamination of spermine

and spermidine, providing the toxic products H_2O_2 and aldehyde(s), able to induce cell death. The cytotoxic effect induced by BSAO and spermine was examined in several human tumor cell lines.

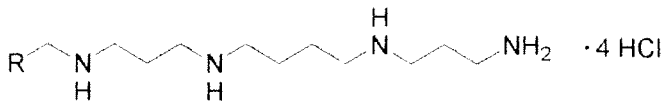
In buffer phosphate 0.01M, it was observed an improvement of both kinetic parameters of the analogs if compared with those obtained in the same experimental conditions for spermine and spermidine. Such as effect was more evident on the K_m values than k_c ones (2).

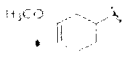
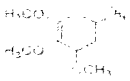
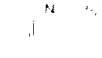
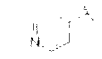
Since the cytotoxic studies using BSAO in the presence of polyamines analogs will be carried out in medium, a kinetic observations were also performed in buffer phosphate 0.01M, at pH 7.6. In these conditions it was observed, in the most of these molecules, a decreasing of K_m with a corresponding increasing of k_c. (Table 1).

The improvement of the kinetic parameters at pH 7.6 is probably due to a better interaction between amine-oxidase with the substrates, constituted by polyamine analogs, that leads to the formation of the Schiff base (3).

As future perspective, these molecules will be used alone or in combination with BSAO, with the aim to determine their cytotoxic effect on several human tumor cell lines, that might represent a new approach in anti-cancer therapy.

Table 1. *Kinetic parameters of some polyamine analogs determined in buffer phosphate 0.01 M.*



Substrate R =	V_{max} (μM/s) BP 7.4	V_{max} (μM/s) BP 7.6	k_c (s^{-1}) BP 7.4	k_c (s^{-1}) BP 7.6	K_m (μM) BP 7.4	K_m (μM) BP 7.6
	0.189	0.255	0.38	0.52	22	18
	0.1	0.13	0.21	0.25	21	7.2
	0.42	0.64	0.85	1.29	20.8	7.2
	0.38	0.45	0.76	0.91	18	12
	0.30	0.39	0.61	0.79	14	7
	0.34	0.29	1.69	0.59	13.6	5
spermine	0.73	0.65	1.83	1.5	26	3.15
spermidine	0.63	0.59	1.45	1.13	114	23.5

References

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2. *Cardillo S, De Iuliis A, Battaglia V , Toninello A, Stevanato R and Vianello F. (2009) Novel copper amine oxidase activity from rat liver mitochondria matrix.Archives of Biochemistry and Biophysics 485, 97–101.*
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