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Short Communication

CATIONIC PORPHYRIN DERIVATIVES AS INHIBITORS OF POLYAMINE CATABOLISM

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Abstract—The effects of six cationic porphyrins on several enzymes involved in polyamine biosynthesis and catabolism have been examined. Both spermidine and spermine synthase were unaffected by the porphyrins at up to 2 mM. By contrast, ornithine and *S*-adenosylmethionine decarboxylase were inhibited by the nickel and cobalt derivatives of *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)porphyrin (T4MPyP) with IC_{50} values in the 10–100 μM region. Spermidine/spermine N^1-acetyltransferase (SSAT) and polyamine oxidase (PAO) were highly sensitive to the six *meso*-substituted cationic porphyrins tested, with K_i values as low as 6 nM for SSAT and 85 nM for PAO. These inhibitors may prove useful in defining the structural features of the active site of these enzymes.

Key words: cationic porphyrins; spermidine/spermine N^1-acetyltransferase (SSAT); polyamine oxidase (PAO); enzyme inhibitors; T4MPyP; polyamine biosynthesis

Two enzymes of polyamine back-conversion, SSAT‖ and PAO, have been characterized [1–3] from rat liver and chick duodenum as well as from mouse and human cell lines [4–6]. SSAT catalyzes the formation of N^1-acetylspermine from spermine, while PAO oxidizes N^1-acetylspermine to spermidine. Spermidine is acetylated subsequently to N^1-acetylspermidine by SSAT and then oxidized to putrescine by PAO [7, 8].

The back-conversion pathway has been shown, in certain cases, to be more effective in meeting the putrescine requirement for cellular growth than by *de novo* production from the decarboxylation of ornithine [9, 10]. Although SSAT basal enzyme levels are extremely low and rate-limiting in the back-conversion pathway, this enzyme is highly inducible by a variety of agents [7]. In specific human tumor cell lines, SSAT is induced to high levels by bis-ethylated derivatives of polyamines [11, 12]. These cell lines are of special interest, since associations between SSAT induction and cell growth have been noted. Specifically, certain analogs of spermidine and spermine are strong competitive inhibitors of SSAT. These same analogs are also excellent inducers of the enzymes, and are cytotoxic to melanoma and lung carcinoma cell lines in a manner that correlates with the induction of SSAT [12, 13]. While these associations may be fortuitous, we reason they are strong enough to warrant further examination of other compounds that may prove to be inhibitors of SSAT and other enzymes of polyamine metabolism.

meso-Substituted cationic porphyrins, like polyamines, contain multiple positive charges, which also strongly bind to DNA. Furthermore, the complex nature of the interaction of porphyrins with DNA has been investigated extensively [14–19]. The cationic character of the polyamines and these porphyrins suggested to us that they may have some effect on polyamine metabolism.

Upon screening a family of *meso*-substituted cationic porphyrins, we observed that T4MPyP (Fig. 1), a photosensitizer as well as a DNA ligand [14–18], is also a potent inhibitor of SSAT. This observation led us to examine the inhibitory activity of several cationic porphyrin derivatives against SSAT and other enzymes of polyamine metabolism. It was found that some of these compounds are potent inhibitors of both SSAT and PAO, with only minor effects on the polyamine biosynthetic enzymes, ODC and AdoMetDC, and with no discernible effects on spermidine or spermine synthase.

Materials and Methods

Porphyrin derivatives were gifts from Dr. N. Datta-Gupta (South Carolina State College, Orangeburg, SC). Structures and names of the six porphyrin derivatives are shown in Fig. 1. The synthesis of (Me) (Et) S-TPP (Fig. 1, No. 6) has been described [19]. All porphyrin derivatives were stored in the dark at 2–4°, and the porphyrins were handled under subdued lighting. [^{3}H]-DL-*S*-Adenosylmethionine, used as a substrate in the spermidine and spermine assays, was a gift from Dr. T. Eloranta, Department of Biochemistry, University of Kuopio, Kuopio, Finland.

Human SSAT was purified to homogeneity from MALME-3M melanoma cells, as previously described [4]. A crude extract of L1210 cells was used as the source of ODC, AdoMetDC, spermidine synthase, and spermine synthase. Enzyme incubations were carried out in the dark. SSAT and PAO activities were measured as previously described [4, 5]. ODC and AdoMetDC activities were measured by standard methodologies [20], and both spermidine and spermine synthase activities were determined by method 1 of Raina *et al.* [21].

Results and Discussion

In preliminary experiments, the six cationic porphyrin derivatives shown in Fig. 1 were examined for inhibition of spermi-

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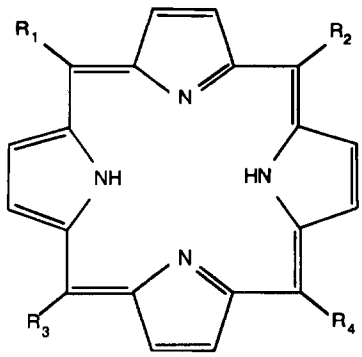
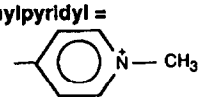
‖ Abbreviations: cis-B4MPyP, *cis-meso*-bis(*N*-methyl-4-pyridiniumyl)diphenyl porphyrin; T4MPyP, *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)porphyrin; CoT4MPyP, cobalt *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)porphyrin; NiT4MPyP, nickel *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)porphyrin; TMAP, *meso*-tetrakis(*N*-trimethylanilinium yl)porphyrin; (Me) (Et)S-TPP, *meso*-tetra[(*p*-ethylmethyl sulfonio)phenyl]porphyrin; SSAT, spermidine/spermine N^1-acetyltransferase; PAO, polyamine oxidase; ODC, ornithine decarboxylase; and AdoMetDC, *S*-adenosylmethionine decarboxylase.



Compound 1 $R_1 = R_2$ = phenyl
$R_3 = R_4$ = 4-N-methylpyridyl =

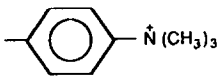


Compound 2 $R_1 = R_2 = R_3 = R_4$ = 4-N-methylpyridyl

Compound 3 Compound 2 + central Ni^{++}

Compound 4 Compound 2 + central Co^{++}

Compound 5 $R_1 = R_2 = R_3 = R_4$ =



Compound 6 $R_1 = R_2 = R_3 = R_4$ =

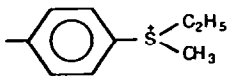


Fig. 1. Structures of *meso*-substituted cationic porphyrins. Compound 1, *cis-meso*-bis(*N*-methyl-4-pyridiniumyl)diphenyl porphyrin, [cis-B4MPyP]. Compound 2, *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)porphyrin [T4MPyP]. Compound 3, nickle *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)porphyrin [NiT4MPyP]. Compound 4, cobalt *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)-porphyrin [CoT4MPyP]. Compound 5, *meso*-tetrakis(*N*-trimethylaniliniumyl)porphyrin [TMAP]. Compound 6, *meso*-tetra-[(*p*-ethylmethyl sulfonio)phenyl]porphyrin [(Me) (Et) S-TPP].

dine and spermine synthase. No inhibition of the synthesis of spermidine from putrescine or of spermine from spermidine was detected at the relatively high concentration of 2 mM (data not shown) for all except B4MPyP, which was evaluated at a somewhat lower concentration because of limited solubility. It is most likely that cationic porphyrins are not inhibitors of either synthase under these conditions, and no further experiments were carried out with these enzymes.

When four other enzymes of polyamine metabolism (ODC, AdoMetDC, SSAT and PAO) were tested, it was possible to establish IC_{50} values for the Ni^{2+} and Co^{2+} chelates of T4MPyP with both ODC and AdoMetDC (Fig. 2). Four other cationic porphyrins, (a) cis-B4MPyP (Fig. 1, No. 1), (b) T4MPyP (Fig. 1, No. 2), (c) TMAP (Fig. 1, No. 5), and (d) (Me) (Et) S-TPP (Fig. 1, No. 6), were not strongly inhibitory against ODC and AdoMetDC since inhibition was only seen at 2 mM. Therefore, the two decarboxylases are at best only weakly affected by these cationic porphyrins. In contrast to the synthases, which were unaffected by the porphyrins, and the decarboxylases, which were only weakly inhibited by these cations, both SSAT and PAO were strongly inhibited by micromolar or submicromolar levels of the porphyrins, as shown in Fig. 2. Indeed, the cobalt–T4MPyP complex (CoT4MPyP) exhibited an IC_{50} against PAO that was substantially below 100 nM, the lowest concentration tested.

A kinetic analysis of the inhibition of SSAT and PAO by the cationic porphyrins was then undertaken. All six cationic porphyrins showed classical competitive inhibition of acetylation of spermidine by SSAT (data not shown). The K_i values, reported in Table 1, ranged from 6 nM for NiT4MPyP to 120 nM for CoT4MPyP. When inhibition of PAO was examined, the non-metallic porphyrins yielded classical Michaelis–Menten inhibition with K_i values ranging from 85 to 1300 nM (Table 1). The metallic porphyrins, although the most potent inhibitors, exhibited complex kinetics, and inhibition constants were not determined.

The data of Table 1 showed appreciable variations in inhibition constants of both enzymes with what are apparently minor changes in the structure of porphyrins. cis-B4MPyP, a dicationic porphyrin, was a relatively inactive inhibitor of SSAT, whereas it was the most active competitive inhibitor of PAO oxidation of N^1-acetylspermine. When two more cationic charges were added to it by substituting two *N*-methypyridinium groups for phenyl groups to form T4MPyP, the change produced a more potent inhibitor of SSAT but a weaker inhibitor (K approximately 5 times greater) of PAO.

Changes in the chemical nature of the side groups of the porphyrin derivatives also had appreciable and differing effects on the ability of the porphyrin to inhibit the two enzymes. Substitution of four trimethylaminophenyl groups (TMAP) in place of four *N*-methylpyridinium groups (T4MPyP) enhanced the potency of the inhibitor against both PAO and SSAT, as seen in Table 1. In contrast, when ethylmethylsulfonio groups were the cationic substitutions [(Mc) (Et) S-TPP, Fig. 1], a major change was observed. While (Me) (Et) S-TPP demonstrated appreciable potency as an inhibitor of SSAT, although the K_i was somewhat higher than that observed for either T4MPyP or TMAP, it was the least effective inhibitor of PAO of the compounds tested here, as seen in both Fig. 2 and Table 1.

Chelation of divalent metal ions in the central porphine ring also gave rise to substantial differences in inhibitory activity. We measured the inhibition with the tetracationic porphyrin T4MPyP and both the nickel and cobalt divalent ion derivatives (Fig. 1). Interestingly, whereas the non-metallic T4MPyP porphyrin had a K_i of 64 nM, the divalent nickel chelate (NiT4MPyP) was found to have a 10-fold lower K_i (6 nM). In contrast, the CoT4MPyP was only one-half as potent as the non-metallo parent, T4MPyP. Examination of the inhibition of PAO by CoT4MPyP and NiT4MPyP demonstrated an unusual type of inhibition that did not yield to conventional kinetic analysis. However, both compounds were more inhibitory than the parent T4MPyP as judged by the data from Fig. 2 and the concentrations used for kinetic analysis (unpublished observations). Based on these studies, there is no obvious explanation for the difference in enzyme inhibition and kinetics seen with the nickel and cobalt T4MPyP.

Table 1. Inhibition constants for SSAT and PAO

Porphyrin derivative	K_i (nM)	
	SSAT	PAO
1. cis-B4MPyP	100	85
2. T4MPyP	64	395
3. NiT4MPyP	6	ND*
4. CoT4MPyP	120	ND
5. TMAP	24	140
6. (Me) (Et)S-TPP	90	1300

The complete name of each porphyrin derivative is given in the legend of Fig. 1.

* ND, not determined.

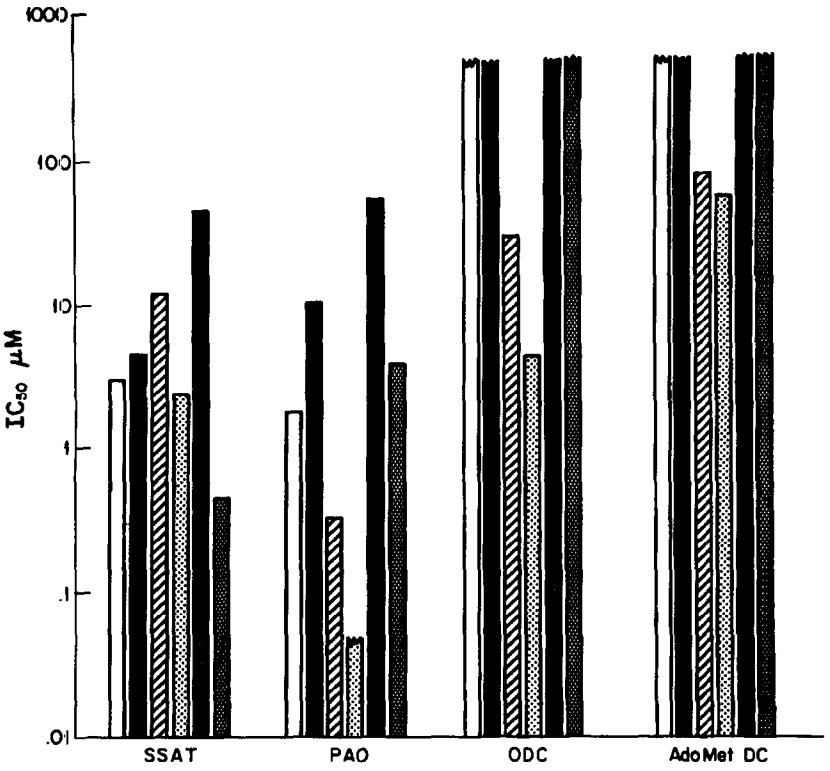


Fig. 2. Inhibition of four enzymes of polyamine metabolism by six *meso*-substituted cationic porphyrins. The inhibitory effects of six cationic porphyrins were determined on two enzymes of polyamine catabolism (SSAT and PAO) and two enzymes of polyamine biosynthesis (ODC and AdoMetDC) as described in Materials and Methods. Inhibition is presented as inhibitor concentration required to decrease activity by 50% (IC_{50}, μM). White bar: cis-B4MPyP. Black bar: T4MPyP. White hatched bar: NiT4MPyP. Light stippled bar: CoT4MPyP. Black hatched bar: TMAP. Dark stippled bar: (Me) (Et) S-TPP.

However, these observations led to the conclusion that the cationic porphyrins, as a class, appear to exhibit strong inhibitory potential against the two enzymes involved in polyamine back-conversion, SSAT and PAO. For this reason, further studies of their chemistry and enzymatic interactions may lead to a greater understanding of the enzymatic mechanisms and the structural features of their active sites.

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