Effect of Platinum(II) Complexes of Benzoic and 3-Methoxybenzoic Acid Hydrazides on *Saccharomyces cerevisiae*

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The inhibitory effect of benzoic acid hydrazide (bah) and 3-methoxybenzoic acid hydrazide (mbah) on *Saccharomyces cerevisiae* strains has been compared to that of their platinum(II) complexes: *cis*-$[Pt(bah)_2X_2]$, *cis*-$[Pt(NH_3)(bah)Cl_2]\cdot 0.5\,H_2O$, *cis*-$[Pt(mbah)_2X_2]$ and *cis*-$[Pt(NH_3)(mbah)Cl_2]$ (X = Cl, Br = I), and *cis*-$[Pt(NH_3)_2Cl_2]$. The minimal inhibitory concentrations for bah and mbah were 5000–20,000 μM whereas for their Pt(II) complexes they were much less (25–800 μM) and did not exceed these of cisplatin (100–800 μM). The activity of the Pt(II) complexes of bah and mbah varied in wide ranges for the different yeast strains tested. Osmotically unstable mutants were found to be more susceptible. The most active complexes were $[Pt(NH_3)(bah)Cl_2]\cdot 0.5\,H_2O$ and $[Pt(NH_3)(mbah)Cl_2]$.

In the search for more efficient analogues of the anticancer agent *cis*-diamminedichloroplatinum (*cis*-platin), special attention has been paid to the use of biologically active compounds as ligands (Hydes and Russel, 1988). Recently Dodoff *et al.* (1994) described the synthesis, characterization and cytotoxic effect of a series of new platinum(II) complexes of benzoic acid hydrazide (bah) and 3-methoxybenzoic acid hydrazide (mbah): *cis*-$[Pt(bah)_2X_2]$, *cis*-$[Pt(NH_3)(bah)Cl_2]\cdot 0.5\,H_2O$, *cis*-$[Pt(mbah)_2X_2]$ and *cis*-$[Pt(NH_3)(mbah)Cl_2]$ (X = Cl, Br, I). The interest in platinum complexes of such ligands was provoked by the literature data about diverse biological activity of carboxylic acid hydrazides and of some of their transition metal complexes: antitumor (Rutner *et al.*, 1974; Tret'yakov *et al.*, 1983; Tret'yakov *et al.*, 1989), mutagenic (Riggin and Schultz, 1986), antimicrobial (Kar *et al.*, 1980; Kutsenko *et al.*, 1980) and antifungal (Zsolnai, 1962; Narang and Singh, 1985; Narang *et al.*, 1990).

In the present study we compare the antiyeast activity of bah and mbah to that of their platinum(II) complexes and cisplatin. We have included in the assay *Saccharomyces cerevisiae* mutants with defective cell wall and their parental strains to find out whether their susceptibility to the test compounds would be different.

The values of the minimal inhibitory concentration (MIC) and the 50%-inhibitory concentration (IC_{50}) of the compounds are presented in Table I. The MIC values of bah and mbah were 5000–20,000 μM, while for their Pt(II) complexes they were more than 50-fold lower (25–800 μM).

The activity of bah was equal to that of mbah against each strain and no difference in susceptibility of the strains, except VY 1160, was observed. The activity of Pt(II) complexes of these ligands, however, varied largely among the strains tested.

The most active were the ammonia-containing complexes, $[Pt(NH_3)(bah)Cl_2]\cdot 0.5\,H_2O$ and $[Pt(NH_3)(bah)Cl_2]$ (MICs ranging from 25 to 200 μM), and the least active were cisplatin $[Pt(bah)_2I_2]$ and $[Pt(mbah)_2I_2]$. No significant differences were observed in the MIC values of $[Pt(bah)_2Cl_2]$, $[Pt(bah)_2Br_2]$, $[Pt(mbah)_2Cl_2]$ and $[Pt(mbah)_2Br_2]$, but it should be noted that against the strain VY 1160, the IC_{50} values of the later two complexes were about 10-fold lower than for the first two.

Although the MIC values of the Pt(II) complexes of bah and mbah were generally lower, they were similar to those of cisplatin, and at the same time they were much lower as compared to the free ligands. This suggests that the new complexes act by a mechanism, common to that of cisplatin, *i.e.* by binding mainly to DNA (Lippert, 1992). Osmotically unstable mutants were observed to be more susceptible to the complexes tested than their respective parental strains. An explanation may be sought in the interaction of the complexes with the altered cell wall and increased availability in the cell, but a supposition that the cell surface may be the site of action of metal complexes (Bunker and James, 1989) is unlikely to apply to platinum complexes. Recently, a protein conferring cis-

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Table I. Inhibitory effect of benzoic acid hydrazides (bah) and methoxybenzoic acid hydrazides (mbah) and their Pt(II) complexes on the growth of *Saccharomyces cerevisiae*, expressed through MIC and IC_{50} in μM.

Compound \ Strain	A364		SY15[c]		E1278		VY481[c]		S288[c]		VY1160[c]	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
bah[a]	20,000	–	20,000	–	20,000	–	20,000	–	20,000	–	5000	–
$[Pt(bah)_2Cl_2]$	200	60	50	30	200	70	20	13	100	50	100	40
$[Pt(NH_3)(bah)Cl]_2 \cdot 0.5H_2O$	200	90	50	30	100	40	20	10	100	60	50	4
$[Pt(bah)_2Br_2]$	200	40	100	70	200	70	50	30	100	40	100	50
$[Pt(bah)_2I_2]$	400	80	200	100	800	120	400	70	400	70	200	30
mbah[b]	20,000	–	20,000	–	20,000	–	20,000	–	20,000	–	5000	–
$[Pt(mbah)_2Cl_2]$	200	20	100	60	200	80	20	6	100	30	50	6
$[Pt(NH_3)(mbah)Cl_2]$	200	60	50	30	200	80	20	10	100	80	50	5
$[Pt(mbah)_2Br_2]$	200	40	50	30	200	50	20	10	100	30	50	5
$[Pt(mbah)_2I_2]$	400	60	200	150	800	200	400	150	400	150	200	20
cis-$[Pt(NH_3)_2Cl_2]$	800	120	100	80	800	100	800	70	400	200	200	10

[a] Benzoic acid hydrazides.
[b] Methoxybenzoic acid hydrazides.
[c] Osmotically unstable mutants of *Saccharomyces cerevisiae*.

platin susceptibility has been isolated from yeast (Brown *et al.*, 1993). Assuming that the Pt(II) complexes of bah and mbah act by a similar mechanism as cisplatin it may be speculated that different susceptibility of the strain is associated with the concentration of the structure specific recognition proteins in the cell.

No correlation was found between the activities of the complexes against yeasts and against Friend leukemia cells, although the IC_{50} values for YV1160 strain were similar to those obtained for Friend leukemia cells, as reported by Dodoff *et al.* (1994).

In conclusion it may be said that participation in Pt(II) complexes dramatically increases the antiyeast activity of bah and mbah and is probably associated with a different mechanism of action. Susceptibility of yeasts to Pt(II) complexes varies widely among the strains. Osmotically unstable mutants are more sensitive than their parental strains.

Experimental

The ligands bah and mbah and *cis*-$[Pt(NH_3)_2Cl_2]$ were prepared according to Struve (1894), Hutton (1955) and Spassovska *et al.* (1981), respectively. The platinum complexes of bah and mbah were synthesized as described by Dodoff *et al.* (1994).

Yeast susceptibility was studied by the minimal inhibitory concentrations (MIC) and the IC_{50} values. DMSO solutions (8–16,000 μM) of the compounds were added to Sabouraud nutrient medium complemented with 10% sorbitol, the DMSO/broth ratio being 1:10. MICs were determined by the twofold broth dilution method (Reiner, 1982), the final innoculum size being 1×10^5 cfu/ml. Readings were made after 48 h of incubation at 30 °C.

IC_{50} were extrapolated from the growth inhibition (I,%) *vs.* concentration curve (Galgiani *et al.*, 1976). I,% was calculated as $(A_c - A_i) : A_c \times 100$, A_c being the optical density at 520 nm of cultures, free from an inhibitor and A_i the respective optical densities of cultures grown in the presence of 800 through 80 and 8 μM of a test compound at the 12th and 16th hour.

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