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

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Pt(II) Complexes, Hydrazides, Yeast Susceptibility

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)2X2], cis-[Pt(NH3)(bah)Cl2] • 0.5H2O, cis-[Pt(mbah)2X2] and cis-[Pt(NH3)(mbah)Cl2] (X = Cl, Br = I), and cis-[Pt(NH3)2Cl2]. The minimal inhibitory concentrations for bah and mbah were 1 0 0.000 0-20.000 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(NH3)(bah)Cl2] • 0.5H2O and [Pt(NH3)(mbah)Cl2].

In the search for more efficient analogues of the anticancer agent cis-diaminedichloroplatinum (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)2X2], cis-[Pt(NH3)(bah)Cl2] • 0.5H2O, cis-[Pt(mbah)2X2] and cis-[Pt(NH3)(mbah)Cl2] (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 anticyeast 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 (IC50) of the compounds are presented in Table I. The MIC values of bah and mbah were 1 0 0.000 0-20.000 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 VY1160, 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(NH3)(bah)Cl2] • 0.5H2O and [Pt(NH3)(mbah)Cl2] (MICs ranging from 25 to 200 μm), and the least active were cisplatin [Pt(bah)2Cl2] and [Pt(mbah)2Cl2]. No significant differences were observed in the MIC values of [Pt(bah)2Cl2], [Pt(bah)Br2], [Pt(mbah)2Cl2] and [Pt(mbah)2Br2], but it should be noted that against the strain VY1160, the IC50 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-
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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Strain</th>
<th>A 364</th>
<th>SY 15$^c$</th>
<th>E 1278</th>
<th>VY 481$^c$</th>
<th>S 288$^c$</th>
<th>VY 1160$^c$</th>
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<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>IC$_{50}$</td>
<td>MIC</td>
<td>IC$_{50}$</td>
<td>MIC</td>
<td>IC$_{50}$</td>
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</tr>
<tr>
<td>bah$^a$</td>
<td>20.000</td>
<td>-</td>
<td>20.000</td>
<td>-</td>
<td>20.000</td>
<td>-</td>
<td>20.000</td>
</tr>
<tr>
<td>[Pt(bah)$_2$Cl$_2$]</td>
<td>200</td>
<td>60</td>
<td>50</td>
<td>30</td>
<td>200</td>
<td>70</td>
<td>20</td>
</tr>
<tr>
<td>[Pt(NH$_3$)$_2$(bah)Cl]$_2$</td>
<td>200</td>
<td>90</td>
<td>50</td>
<td>30</td>
<td>200</td>
<td>70</td>
<td>50</td>
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<tr>
<td>0.5H$_2$O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Pt(bah)$_2$Br$_2$]</td>
<td>200</td>
<td>40</td>
<td>100</td>
<td>70</td>
<td>200</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>[Pt(bah)$_2$I$_2$]</td>
<td>400</td>
<td>80</td>
<td>200</td>
<td>100</td>
<td>800</td>
<td>120</td>
<td>400</td>
</tr>
<tr>
<td>mbah$^b$</td>
<td>20,000</td>
<td>-</td>
<td>20,000</td>
<td>-</td>
<td>20,000</td>
<td>-</td>
<td>5000</td>
</tr>
<tr>
<td>[Pt(mbah)$_2$Cl$_2$]</td>
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<td>20</td>
<td>100</td>
<td>60</td>
<td>200</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>[Pt(NH$_3$)$_2$(mbah)Cl]$_2$</td>
<td>200</td>
<td>60</td>
<td>50</td>
<td>30</td>
<td>200</td>
<td>80</td>
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<td>[Pt(mbah)$_2$Br$_2$]</td>
<td>200</td>
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<td>50</td>
<td>30</td>
<td>200</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>[Pt(mbah)$_2$I$_2$]</td>
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<td>60</td>
<td>200</td>
<td>150</td>
<td>800</td>
<td>200</td>
<td>400</td>
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<tr>
<td>cis-[Pt(NH$_3$)$_2$Cl]$_2$</td>
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<td>100</td>
<td>80</td>
<td>800</td>
<td>100</td>
<td>800</td>
</tr>
</tbody>
</table>

$^a$ Benzoic acid hydrazides.  
$^b$ Methoxybenzoic acid hydrazides.  
$^c$ Osmotically unstable mutants of *Saccharomyces cerevisiae*.

Platinum 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 YV 1160 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$_3$)$_2$Cl]$_2$ 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 inoculum 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$_c$×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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