Buffering Effects of Benzimidazole in Absorption of Potassium by Excised Barley Roots 1, 2, 3

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Introduction

Benzimidazole (BZ) enhancement of ion uptake was recently reported by Klingensmith (11) and Klingensmith and Norman (12). Their experiments were conducted with excised barley roots at rather high root to solution ratios (7) without added buffer. Potassium uptake was determined by difference, without the complications due to the presence of other ions, but in such systems there is apparently a progressive increase in acidity during the experimental period, which results in a change in the rate of uptake of the cation. The effect of hydrogen ion concentration on K+ absorption by excised barley roots has been discussed in detail by Jacobson et al. (7, 8, 9) and by Nielsen and Overstreet (14). These workers have reported that the absorption of K+ decreases markedly in the pH range from 6 to 4 and that tissue injury probably occurs at lower pH values followed by loss of cellular constituents to the external medium.

Hofmann (6) in a discussion of the chemical properties of the benzimidazoles points out that these compounds are amphoteric. The acidic property of BZ is reflected in its ability, under quite specific conditions, to form rather stable salts and complexes with certain metals, such as Ag, Cu, Cd, Ni, Co, Hg, and Zn. In addition, crystalline BZ salts of Li, Na, K, and Ba have been prepared; however, these are very unstable and hydrolyze upon exposure to water with regeneration of BZ. Substitution of the imino hydrogen on the imidazole ring eliminates the acidic property.

The basic characteristic of BZ results from the ability of the pyridine (tertiary nitrogen) nitrogen of the imidazole ring to accept a proton and thus become ionized as a cation. In this respect, BZ is a weak base that is able to act as an effective buffer.

The present study was conducted to investigate further the apparent enhancement of the uptake of K+ by BZ in the light of the recognition of the strong buffering property of this compound.

Materials & Methods

Excised barley roots (Hordeum vulgare L., var. Atlas 54) from 7 day old seedlings were used in this study. Seed from the 1959 crop had been stored at 0 C to 3 C prior to use in 1961. Root material was cultured according to the method of Romberger (15). The roots were excised by cutting immediately below the supporting screen and were handled gently to minimize mechanical injury. The excised roots were washed several times with cold distilled water and the excess water removed in a basket centrifuge. The roots were allowed to remain intact after excision with no further cutting or segmentation. The elapsed time between excision and treatment never exceeded 15 minutes. Samples of roots (7.5 g fr wt) were placed in polystyrene vessels containing 200 ml of the desired solutions and aerated continuously during the absorption period. All experiments were conducted at 25 C. The concentration of K2SO4 used throughout this study was 1 x 10^{-3} M. Benzimidazole was obtained from Eastman Kodak Co., Rochester, N. Y.

The pH of solutions throughout the K+ absorption or uptake period was maintained close to 6.5 by periodic additions of small quantities of analytical grade Amberlite IRA-400 anionic exchange resin in OH\(^-\) form. The resin was supplied by the Rohm and Haas Co., Philadelphia, Pa., in the Cl\(^-\) form and converted to the OH\(^-\) form by treatment with excess 4\% NaOH solution, until chloride free. With 7.5 g roots in 200 ml K2SO4 solution a total of approximately 0.25 g dry weight of resin was added in six or seven increments during a 6 hour period as required. Uniform control of pH required that most of the resin be added in three or four increments during the first hour of absorption with small and less frequent increments thereafter. The pH changes were followed closely with a Beckman Zeromatic pH meter. This proved to be a satisfactory method for controlling pH and eliminated the addition of KOH or other inorganic bases to the systems to prevent an increase in acidity. Additional details of this experimental technique will appear elsewhere.

Two series of experiments were performed; i.e., one in which excised barley roots were placed immediately in the desired K2SO4+BZ solutions and another in which roots were pretreated with BZ prior to transfer to the uptake solutions.

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In the pretreatment experiments, the excised roots were placed for 3 hours in BZ solutions or distilled water with continuous aeration, after which they were rinsed several times with distilled water before transfer to solutions of K$_2$SO$_4$+BZ or K$_2$SO$_4$ alone for a 3 hour K$^+$ absorption period. During the pretreatment phase, pH control was necessary to prevent a progressive decrease in pH due to root activity. This was especially true of excised root systems in distilled water. Both these and the BZ treated roots were maintained at pH 6.0 (the pH of fresh distilled water.) The BZ stock solution (1 x 10$^{-3}$ M) initially pH 6.8, was adjusted to 6.0 by addition of 0.18 N H$_2$SO$_4$. At intervals, as necessary, the water or BZ solution was decanted off the roots and replaced on the same schedule to maintain the pH at 6.0. Only during the subsequent K$^+$ absorption period was the resin addition technique followed.

At the end of the absorption period the solutions were decanted and analyzed for potassium by flame photometry using a Beckman model DU spectrophotometer. Potassium uptake was determined by difference between the initial and final content of the ambient solution.

### Results

Previous work (11,12) indicated enhancement of K$^+$ absorption by excised barley roots in the presence of BZ concentrations from 3 x 10$^{-3}$ M to 1 x 10$^{-4}$ M with a peak in the region of 1 x 10$^{-4}$ M where K$^+$ absorbed in 3 hours was almost double that from K$_2$SO$_4$ alone. These observations were repeated and substantiated as shown in table I. However, it appeared possible that the buffering action of

<table>
<thead>
<tr>
<th>Molar conc of BZ</th>
<th>3 x 10$^{-3}$</th>
<th>1 x 10$^{-3}$</th>
<th>3 x 10$^{-4}$</th>
<th>1 x 10$^{-4}$</th>
<th>3 x 10$^{-5}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>K$^+$ uptake at 3 hr</td>
<td>105</td>
<td>190</td>
<td>135</td>
<td>112</td>
<td>95</td>
</tr>
</tbody>
</table>

* Results are means of three experiments with three replicates in each.

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Fig. 1 (Left). Decrease in pH as a result of K$^+$ absorption by excised barley roots. 7.5 g root, fresh weight in 200 ml 1 x 10$^{-3}$ M K$_2$SO$_4$ at 25 C for six hours. Upper curve—K$_2$SO$_4$ plus 1 x 10$^{-3}$ M Benzimidazole (BZ). Lower curve—K$_2$SO$_4$ alone. Each point is the mean of three replicates.

Fig. 2 (Right). Cumulative potassium absorption by excised barley roots as influenced by Benzimidazole. 7.5 g root, fresh weight in 200 ml 1 x 10$^{-3}$ M K$_2$SO$_4$ (K) at 25 C for 6 hours. Benzimidazole (BZ), when present, 1 x 10$^{-3}$ M. Upper two curves—pH of solutions maintained close to 6.5 by periodic additions of Amberlite anionic exchange resin IRA-400 in OH$^-$ form (R). Lower two curves—pH of solutions uncontrolled; pH of K$^+$ + BZ was initially 6.8 and K, 5.9. Subsequent changes were as noted on the respective curves. Each point is the mean of three replicates.
BZ prevented the rapid decrease in pH that occurred in the K₂SO₄ controls, and that a BZ concentration of 1 × 10⁻³ M did not permit the pH to fall below 5.5 in six hours. The strong buffer action of BZ, therefore, masked its real effect on excised barley roots, which is a repression of K⁺ absorption, in line with other repressive effects on growth described by Klingensmith (10).

Figure 1 shows the decrease in pH as a result of K⁺ absorption by 7.5 g roots from 200 ml of 1 × 10⁻³ M K₂SO₄ with and without BZ. The pH of solutions was not controlled. The effectiveness of 1 × 10⁻³ M BZ as a buffer in the uptake solution is noted. During a 6 hour absorption period, pH of the K₂SO₄+BZ solutions shifted from an initial reading of 6.85 to a final reading of 5.45, while solutions without BZ shifted from 5.90 to 3.95. The very rapid downward shift in pH during the 1st hour is in agreement with observations of Jacobson et al. (7) on KBr solutions.

In subsequent experiments downward shifts in pH were minimized by adding Amberlite IRA-400 anionic exchange resin in OH⁻ form. Frequent pH readings were taken throughout the absorption period and pH was maintained close to 6.5 by periodic addition of moist resin to the bottom of the containers. The pH differences between replicates did not exceed 0.2 pH units at any time.

The two upper curves of figure 2 show that when pH is controlled the effect of 1 × 10⁻³ M BZ on excised barley roots is a repression of K⁺ absorption and not an enhancement as previously reported. These cumulative K⁺ absorption curves are typical of results obtained when pH was controlled by the resin technique described. The two lower curves are representative of K⁺ absorption when pH was uncontrolled and shifted downward, as a result of root activity and the unbalanced uptake of K⁺ and SO₄⁻. Initial pH values for the two lower curves are approximately the same as shown in figure 1.

In similar experiments, summarized in table II, the effect of BZ on K⁺ absorption was studied at lower BZ concentrations. A definite repression was observed at 1 × 10⁻⁴ M BZ, with only a slight repression at 1 × 10⁻⁵ M BZ, and no detectable repression at lower concentrations.

### Table II

<table>
<thead>
<tr>
<th>Molar conc of BZ</th>
<th>K uptake at 3 hr as % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 × 10⁻⁸</td>
<td>73(12)</td>
</tr>
<tr>
<td>1 × 10⁻⁴</td>
<td>90(9)</td>
</tr>
<tr>
<td>1 × 10⁻⁵</td>
<td>99(9)</td>
</tr>
<tr>
<td>1 × 10⁻⁷</td>
<td>100(6)</td>
</tr>
</tbody>
</table>

* Figures are means of values obtained from the number of replicates shown in parenthesis.

In previous studies (11, 12) some specific action of BZ in enhancing K⁺ absorption was inferred from experiments in which excised roots were pretreated for 3 hours with 1 × 10⁻³ M BZ, followed by several washings with distilled water prior to being placed in the K₂SO₄ solutions. These experiments were repeated with pH control both during pretreatment and in the subsequent K⁺ absorption period. After establishing consistent repression of K⁺ absorption by roots in BZ solutions, it was surprising to find a small but significant increase in K⁺ absorbed from K₂SO₄ solution alone by roots previously exposed to BZ. The results of three such experiments are summarized in table III. It should be noted that these data are for a 3 hour absorption period following 3 hours of BZ pretreatment. When the uptake period was extended to 6 hours following 3 hours of BZ pretreatment (>9 hours from excision) the enhanced uptake by the BZ pretreated roots was greater than at 3 hours, but the differences between replicates also became greater. The statistical tests of significance of the data in table III apply only to the effect of the pretreatment. When BZ was present in the uptake solutions its effect on K⁺ absorption was always repressive, although not strongly so.

### Table III

<table>
<thead>
<tr>
<th>Pretreatment solution</th>
<th>Uptake solution</th>
<th>mg K Absorbed by 7.5 g roots*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water K₂SO₄+BZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.57</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>BZ K₂SO₄+BZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.05**</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Water K₂SO₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.68</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>BZ K₂SO₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.98</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

* Figures are means of values obtained from the number of replicates shown in parenthesis. Both BZ and K₂SO₄ solutions were 1 × 10⁻³ M.
** Significantly different from the value above, at the 5% level.
*** Significantly different from the value above, at the 1% level.

In the light of the observations reported herein it would appear to be pertinent to re-examine the various suggestions regarding possible mechanisms of action of BZ on microorganisms and higher plants. Benzinidazole was suggested to act as a purine antimetabolite by Woolley (17), who reported a reversal of BZ-inhibited growth of *Saccharomyces cerevisiae* with guanine and adenine. However, subsequent work by Galston et al. (3) with pea epicotyls, by Hillman (5) with *Lemma minor*, and by other workers has failed in demonstrating clearly an anti-purine role for BZ.

### Discussion

In the light of the observations reported herein it would appear to be pertinent to re-examine the various suggestions regarding possible mechanisms of action of BZ on microorganisms and higher plants. Benzinidazole was suggested to act as a purine antimetabolite by Woolley (17), who reported a reversal of BZ-inhibited growth of *Saccharomyces cerevisiae* with guanine and adenine. However, subsequent work by Galston et al. (3) with pea epicotyls, by Hillman (5) with *Lemma minor*, and by other workers has failed in demonstrating clearly an anti-purine role for BZ.
Galston et al. (3) reported that \(1 \times 10^{-3}\) M BZ specifically affected the action of indoleacetic acid (IAA). They suggested this specific role for BZ because no effect of BZ was observed in the absence of IAA. Inhibition of cell elongation caused by BZ was only partially reversed by adenine, guanidine, and other purines. Heath and Clark (4) have suggested that IAA normally interacts with metal ions in producing its physiological effects, and if this is the case, antagonism between IAA and BZ might be due to mutual competition for metal ions.

Hillman (5) has attributed the growth inhibition effects of BZ on *Lemna minor* mainly to a BZ-sequestration of copper within the plant and to the resultant disturbance in copper metabolism. However, he was not able to reverse the effects of BZ by adding increased levels of Cu, Zn, Fe, or Mn. As opposed to this finding McCorquodale and Duncan (13) have reported that the remarkably similar growth inhibition effects on *Vicia faba* by BZ, imidazole, and histamine could be prevented by the addition of certain metals, notably Mn. Since these three compounds are amines (all have an unsubstituted imidazole ring) and known to be chelating agents, the formation of metal chelate structures would be a plausible mode of action of BZ; however, evidence pointing to the formation of strong chelate structures involving BZ was not obtained.

Another mechanism of action which might apply to BZ is that proposed by Eyring and Dougherty (2). They suggest that certain amines, such as histamine, act as pump substances by formation of weak coordination complexes with certain metal ions, such as sodium, and then move across biological membranes in opposition to electrical potentials. They theorize that these amines may pump metal ions into or out of cells depending on specific conditions and that the resulting disturbance of normal membrane potentials then leads to a disruption of sensitive cells. However, potassium is not a metal ion that would be likely to be pumped in this way.

From the discussion so far, it is apparent that the exact mechanism of action of BZ is not clear and that BZ may possibly act in a number of ways to produce its inhibitory effects. The fact that BZ is amphoteric, and becomes charged as a cation (pK 5.5) in fulfilling its role as a buffer, emphasizes that the activity and some of the subsequent effects of the compound may depend greatly on pH. This does not seem to have been given consideration except by Hillman (5) and Alonso (1). Hillman, while observing the effect of BZ on *Lemna minor*, noted at pH 4.2 that a concentration of \(6.78 \times 10^{-3}\) M BZ was necessary to obtain the same response given by \(8.5 \times 10^{-4}\) M BZ at pH 6.2. Similar observations were made by Alonso in studying the effect of pH on BZ-inhibited growth of *Saccharomyces cerevisiae*. At a constant level of BZ (3.4 \(\times 10^{-2}\) M) he found the degree of inhibition at pH 4.5, 5.0, and 5.6 to be 72, 49, and 93\%, respectively. Hofmann (6) has noted the pK of BZ to be approximately 5.5, indicating that BZ is most active in the uncharged state, as would be expected for weak organic bases by the findings of Simon and Beevers (16). The foregoing discussion would indicate that BZ penetrates biological membranes most readily in the uncharged state.

It is our conclusion that in previous reports of BZ-enhanced K\(^+\) absorption by excised barley roots, pH changes were mainly responsible for the results obtained and that the repressive effect of BZ on K\(^+\) absorption was masked by its buffering action in systems where pH was not controlled. When the pH is controlled the effect of BZ concentrations greater than \(1 \times 10^{-3}\) M presented to roots simultaneously with the potassium salt is one of a repression or inhibition of K\(^+\) absorption. However, it was interesting to establish a small but consistently significant increase in K\(^+\) absorption by roots previously treated with BZ even when external pH conditions were fully controlled. Although this might suggest some specific effect, it is possible that the buffering action of this compound might again be involved. During the BZ pretreatment of excised barley roots at pH 6.0, the BZ concentration in the free space would be in equilibrium with that outside, and in addition some BZ would be expected to enter root cells. There is no direct evidence for this with excised roots, but the morphological changes observed (10) with intact plants could only be explained on the basis of ready entry and transport. On terminating the pretreatment period the superficial rinsings of the roots would not be likely greatly to reduce the BZ concentration in the free space, and would certainly not remove BZ from root cells. In the subsequent uptake period, then, BZ might exert effects both in the free space and in root cells. In the free space presumably its effect would be limited to a buffering action, which in the immediate proximity of absorption sites might disproportionately affect K\(^+\) uptake. This would be of relatively short duration because the concentration of BZ in the free space would decline due to equilibration with the external solution. However, in the root cells there might be some protracted action involving a change in the pH of cellular fluids, possible coordination with metal ions resulting in changes in electrical potentials, or more specific effects on the respiratory or accumulatory systems. Further experimentation with other compounds that may have buffering action is contemplated.

**Summary**

Cation absorption by excised barley roots has been reported to be enhanced by benzimidazole (BZ). This apparent enhancement, however, is due to the activity of this compound as a buffer. When the pH of the K\(_2\)SO\(_4\) solutions in which excised barley roots are placed is fully controlled, the effect of BZ concentrations greater than \(1 \times 10^{-5}\) M is one of a repression or inhibition of K\(^+\) absorption. Its mode of action has not been determined. A concentration of \(1 \times 10^{-3}\) M reduces K\(^+\) uptake approximately 25\%.
Pretreatment of roots with \(1 \times 10^{-3}\) M BZ causes a small but consistently significant increase in subsequent \(K^+\) absorption. Internal buffering by this compound may be responsible for this effect.

**Literature Cited**


