The presence of the enzyme lipoxidase in alfalfa has been reported (3, 5, 6). However, the properties of this postulated alfalfa lipoxidase are quite different from the well-known properties of soybean lipoxidase. Strain (5) showed that the alfalfa enzyme acts on oleic and ricinoleic acids, however it is known that soybean lipoxidase acts only on linoleic, linolenic and arachidonic acids. Mitchell and Hauge (3) showed that the alfalfa enzyme was partially or completely inactivated by copper sulfate, basic lead acetate, sodium cyanide, thiourea and sodium fluoride. These reagents in relatively high concentrations have no effect on soybean lipoxidase. Recently, Walsh and Hauge (9) determined optimum temperature and pH and the cyanide inhibition of alfalfa suspensions. After comparing their findings with the properties of soybean lipoxidase, they came to the conclusion that the two enzymes are widely different. It is the purpose of this study to establish the identity of the alfalfa enzyme as a lipoxidase and to determine some of its important properties.

The enzyme suspensions were prepared by pressing the leaves of fresh mature alfalfa (California common variety) in a hydraulic press. All experiments were done on this juice. Attempts at further purification were unsuccessful. Highly purified ammonium linoleate was the substrate. Reaction rates were measured by the standard Warburg techniques already described (4).

In the first series of experiments, it was found that linolate alone, linolate + heated alfalfa juice and unheated alfalfa juice alone did not absorb any appreciable amounts of oxygen. The oxygen absorption value of 7.7 μl/min in the case of linolate + alfalfa juice, therefore, suggests the presence of an enzyme system. Significant is the fact that ammonium oleate in the same concentration as linolate and treated exactly the same way was not oxidized when reacted with the alfalfa juice. These observations indicate the presence of a lipoxidase similar to soybean lipoxidase which specifically catalyses the oxidation of unsaturated fatty acids having two methylene interrupted double bonds.

In order to further compare the properties of the alfalfa enzyme with soybean lipoxidase, inhibitor and antioxidant studies were performed. Though an oxidizing enzyme, soybean lipoxidase is not known to possess a prosthetic group (1). Like soybean lipoxidase, the lipoxidases from mung beans (Phaseolus aureus), peas, wheat, peanuts and urd beans (Phaseolus mungo) are not inhibited by metal inhibitors or sulphydryl agents (Siddiqi, unpublished results; 4).


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Table I
Inhibition by Antioxidants of Alfalfa Enzyme Catalysis

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Concentration</th>
<th>Inhibition of the Initial Reaction Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 x 10⁻⁴ M</td>
<td>%</td>
</tr>
<tr>
<td>Nordihydroguaiaretic acid</td>
<td>0.1</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Propyl gallate</td>
<td>1</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>91</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>64</td>
</tr>
<tr>
<td>a-Tocopherol</td>
<td>10</td>
<td>73</td>
</tr>
</tbody>
</table>

Accordingly, the effect of cyanide, azide, fluoride, diethylthiocarbamate and p-chloromercuribenzoate as a final concentration of 1 x 10⁻³ M was determined on the rate of oxygen absorption of the linolate-alfalfa juice reaction mixture. None of the above reagents inhibited the oxygen absorption appreciably. These inhibitor studies indicate that alfalfa enzyme, like other known lipoxidases, does not give direct evidence of a prosthetic group similar to those occurring in most oxidase or peroxidase enzymes. Results of the effect of antioxidants, nordihydroguaiaretic acid, propylgallate and a-tocopherol on the reaction velocity are given in Table I. Here again a striking similarity of the alfalfa enzyme with soybean lipoxidase (8) is observed.

Further evidence for the identity of the alfalfa enzyme as a lipoxidase came from a determination of peroxides and conjugated dienes in a sample of linolate which had been oxidized by the alfalfa enzyme. The peroxide concentration was determined by the method of Lundberg and Chipault (2). The amount of conjugated dienes was determined by measuring absorption at 233 mμ. Based on the E₅₀ value of 27, 400 (7), 1.3 x 10⁻² millimoles of conjugated dienes were formed. The peroxide determination yielded a value of 1.4 x 10⁻² millimoles of peroxide produced. Since the ratio of these values is near unity, this indicates that the linolate oxidation products of alfalfa lipoxidase catalysis are similar to those of soybean lipoxidase catalysis.

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LITERATURE CITED


PROMOTION OF LEAF EXPANSION BY KINETIN AND BENZYLAMINOPURINE1,2,3,4

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Kinetin, 6-(2-furfuryl)-aminopurine which was recently isolated from autoclaved deoxyribonucleic acid, has been shown to interact with indoleacetic acid (IAA) in controlling root and bud formation in tobacco pith callus tissue cultures (1). Kinetin and some of its analogs (kinins) also appear to regulate budding in moss (Tortella caespitosa) and root growth in tomato (Lycopersicum esculentum Mill.) (2, 3). Although it has been shown that light and the cobaltous ion exert control over oat coleoptile growth (4, 5) and over leaf expansion (4, 6, 7) the regulation of these processes by kinetin or its analogs has not been demonstrated. The experiments reported below were undertaken to determine the effect of kinins on leaf expansion and whether they interact in some manner with red and far-red light and cobalt to control this process.

Disks 5 mm in diameter were taken from etiolated primary leaves of Burpee dwarf stringless greenpod beans and were grown in Petri dishes on filter paper containing 5 ml of Miller’s media (8) modified to include pH 5.6 buffer consisting of 5 gm of potassium-sodium tartrate and 1 gm of potassium dihydrogen phosphate per liter of solution. The cobaltous ion was added as cobaltous nitrate at a concentration of 20 ppm which appears to be optimum under our conditions (unpublished data). All manipulations prior to light treatment were carried out under a green safelight. Approximately 1600 foot-candle-minutes (FCM) of red light (6000 to 7000 Å, maximum at 6400 Å) were obtained by filtering fluorescent light through two layers of DuPont 300 MSC red cellophane. Four hundred FCM of far-red (greater than 6800 Å, approximately 40 % transmission at 7300 Å, 60 % at 8000 Å) were secured by passing light from an incandescent source through two layers of red and two layers of dark blue DuPont 300 MSC cellophane. This quality of light was essentially that used previously (4). After the light treatment, the disks were placed in darkness at 25°C until harvested. Results are expressed in terms of millimeters increase in diameter after 48 hours of growth.

The results in table I show the effect of two different kinins with or without cobalt on the expansion of etiolated bean leaf disks given red light or darkness. It is apparent in every case that the kinins used in these experiments promote leaf expansion above the controls until an inhibitory concentration is reached. The concentration for maximum growth appears to be in the region from 0.1 to 1.0 µg/ml. Either cobalt or these kinins promote leaf expansion more than does red light; furthermore, the response to light is essentially additive to that for cobalt or for the kinins. In nearly every instance, however, when cobalt and the kinins are given together in either light or dark the increase in diameter is considerably less than additive. For example, the increase due to cobalt alone in darkness is 1.09 mm (1.73-0.64). The response due to 6-benzylaminopurine at a concentration of 0.3 micrograms per liter in the dark is 1.15 mm (1.79-0.64). If the growth due to cobalt and to 6-benzylaminopurine were additive, the disks should have grown 2.14 mm more than those in basal medium whereas they actually grew only 1.54 mm more than the con-