Trypsin Inhibitor Activity in the Field Bean (*Vicia faba* L.)

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*(Accepted for publication February 1972)*

In *vitro* evidence is presented for the presence of a heat-labile inhibitor of trypsin in both cotyledon and testa of the field bean (*Vicia faba* L.). Inhibitor activity is destroyed by heating at 110 °C for 40 min. The inhibitor has, *in vitro*, approximately one fifth of the strength of an extract of raw soyabeans.

1. Introduction

Wilson and McNab\(^1\) reported an increase in live-weight and a reduction in pancreas size of chicks when field beans (*Vicia faba* L.) were autoclaved at 120 °C for 30 min. Such effects may be due to the presence in the raw field bean of a trypsin inhibitor analogous to those found in the soyabean and other legumes. Information on the presence of trypsin inhibitors in the field bean and on growth of animals fed on heat-treated field beans is meagre and contradictory. Some of the contradiction arises because the Indian bean *Dolichos lablab* has the common name field bean and some research into anti-nutritive factors in this bean has been reported under that common name.\(^2,3\)

In this study the name field bean will apply only to *Vicia faba*.

Borchers and Ackerson\(^4\) found no trypsin inhibitor activity (t.i.a.) in field beans. In further work\(^5\) rat growth was significantly increased from 1.58 to 2.12 g/day when field beans were autoclaved at 121 °C for 30 min, although the field bean sample used showed no t.i.a. Haemagglutinating activity in the field bean was reduced by autoclaving at 121 °C for 30 min,\(^6\) but the initial activity was low and unlikely to explain the growth response to autoclaving reported elsewhere.\(^1\) Nitsan,\(^7\) contrary to other findings,\(^1\) reported that growth rate and weight gain per unit of food eaten were both reduced when rats were fed on autoclaved field beans and that trypsin and chymotrypsin inhibitors were not destroyed by heat treatment.

In this study the sample of field beans used in previous chick growth studies\(^1\) is examined *in vitro* for evidence of t.i.a.

2. Experimental

Samples of field bean and soyabean meals were assayed for t.i.a. by the method of Erlanger, Kokowsky and Cohen\(^8\) as modified by Sambeth, Nesheim and Serafin.\(^9\) Samples were prepared by grinding in a laboratory mill until the test material passed a

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45 679
44-mesh sieve. Ten grams of the resulting flour were suspended in 100 ml of 0.0025 M-HCl and stirred for 30 min. The suspension was centrifuged at 1500 g for 30 min and the precipitate was discarded. After 20 min at 23.5 °C the inhibition of trypsin digestion of N-benzoyl DL-arginine p-nitroanilide hydrochloride (BAPA) in the presence of bean extracts was estimated by measuring absorbance of test solutions at 410 nm on an SP 600® Spectrophotometer coupled to an SP 22® Recorder operating in the logarithmic mode. Control tubes containing a blank extract were used as datum for each assay.

Isolates with high t.i.a. values were extracted by the methods of both Sohonie and Ambe* and Garlich and Nesheim¹⁰ using defatted field bean meal. The resultant bean protein solution was dialysed against water for 48 h before freeze-drying.

**TABLE 1. Absorbance (×10³) of assay mixtures containing blank or field bean extracts**

<table>
<thead>
<tr>
<th>Trypsin concentration (mg/100 ml)</th>
<th>0</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance of mixture with blank extract</td>
<td>6.4</td>
<td>19.6</td>
<td>30.6</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td></td>
<td>13.2</td>
<td>24.2</td>
</tr>
<tr>
<td>Absorbance of mixture with bean extract</td>
<td>30.0</td>
<td>30.1</td>
<td>30.0</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td></td>
<td>0.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Chromatographic analysis of an isolate, prepared by the method of Garlich and Nesheim,¹⁰ was carried out on a column of DEAE cellulose using a batch elution technique. Aliquots (10 ml) were collected from the column and analysed for protein before being bulked into fractions and freeze-dried. Trypsin inhibitor activity of the fractions was determined as described above.

**3. Results**

The effects of field bean extracts on trypsin digestion are shown in Table 1. The field bean extract inhibited trypsin digestion as absorbance did not rise with increase in trypsin concentration. Because of the high “blank” value of bean extract the validity of absorbance comparisons was tested by addition of p-nitroaniline to blank extracts. Increase in absorbance is unaffected by the level of background colour.

Extracts of raw field beans and field beans autoclaved at 110 °C for 20 or 40 min are compared in Table 2. An extract from field beans which were autoclaved for 20 min inhibited trypsin digestion of BAPA, but autoclaving for 40 min destroyed inhibitor activity.

A sample of field beans was separated manually into cotyledon and testa. The effect of extracts of these fractions on trypsin activity is shown in Table 3. The extract of bean testa was too deep in colour for direct assay and was decolorised by addition of saturated barium hydroxide solution until the extract reached pH 7 and then centrifuged

*Pye-Unicam, Ltd, Cambridge.
at 1500 g for 10 min. Trypsin was inhibited completely by cotyledon extract but only partially by testa extract. The cotyledon thus seems to have greater t.i.a. per unit weight than the testa. The use of barium hydroxide for clarification of the testa solution did not affect trypsin activity.

### Table 2. Absorbance (×10²) of assay mixtures containing blank extract or extracts of field beans autoclaved for 20 or 40 min at 110 °C

<table>
<thead>
<tr>
<th>Trypsin concentration (mg/100 ml)</th>
<th>0</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance of blank mixture</td>
<td>6.8</td>
<td>17.0</td>
<td>28.4</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td>—</td>
<td>10.2</td>
<td>21.6</td>
</tr>
<tr>
<td>Absorbance of mixture with extract from beans autoclaved for 20 min</td>
<td>13.0</td>
<td>12.7</td>
<td>13.1</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td>—</td>
<td>—0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Absorbance of mixture with extract from beans autoclaved for 40 min</td>
<td>12.2</td>
<td>23.0</td>
<td>32.5</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td>—</td>
<td>10.8</td>
<td>20.3</td>
</tr>
</tbody>
</table>

### Table 3. Absorbance (×10²) of assay mixtures containing blank extract or extracts of field bean testa and cotyledon

<table>
<thead>
<tr>
<th>Trypsin concentration (mg/100 ml)</th>
<th>0</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance of mixture with blank extract</td>
<td>12.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td>—</td>
<td>8.0</td>
</tr>
<tr>
<td>Absorbance of mixture with cotyledon extract</td>
<td>14.0</td>
<td>13.8</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td>—</td>
<td>—0.2</td>
</tr>
<tr>
<td>Absorbance of mixture with testa extract</td>
<td>25.4</td>
<td>31.2</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td>—</td>
<td>5.8</td>
</tr>
<tr>
<td>Absorbance of mixture with blank extract</td>
<td>4.0</td>
<td>25.8</td>
</tr>
<tr>
<td>Absorbance of mixture with blank extract and barium hydroxide</td>
<td>4.5</td>
<td>26.0</td>
</tr>
</tbody>
</table>

**3.1. Trypsin inhibitor isolate**

Preparation of an isolate by the method of Sohonie and Ambe² yielded 1 g of fine white powder from 1 kg of field bean meal. The material was very soluble in water and had a nitrogen content of 1.06%.

The method of Garlich and Nesheim¹⁰ yielded 52 g of isolate from 10 kg of field beans. This preparation contained 8.17% of nitrogen. The isolate exhibited considerable t.i.a. For comparative purposes, extracts of raw and autoclaved (30 min at 110 °C)
field bean and soyabean meals were prepared from the same weight of beans and were
diluted until each reduced absorbance of assay mixtures by a similar amount. This
comparison is shown in Table 4. Under the conditions of assay in this study the t.i.a. of
the field bean isolate is approximately half that to be expected from assay with the
whole bean and the soyabean has a t.i.a. five times that of the field bean.

Table 4. Absorbance ($\times 10^3$) of assay mixtures containing inhibitor
isolate or extracts of soyabean meal or raw or autoclaved field bean
meal

<table>
<thead>
<tr>
<th>Trypsin concentration (mg/100 ml)</th>
<th>0</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank extract</td>
<td>8.3</td>
<td>15.8</td>
<td>22.4</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td>7.5</td>
<td>14.1</td>
<td></td>
</tr>
<tr>
<td>Raw field bean extract</td>
<td>14.5</td>
<td>16.8</td>
<td>19.2</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td>2.3</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Autoclaved field bean extract</td>
<td>15.4</td>
<td>20.0</td>
<td>25.1</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td>4.6</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>Soyabean extract diluted 1:1</td>
<td>15.0</td>
<td>16.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td>1.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Isolate (concentrated×2)*</td>
<td>9.2</td>
<td>11.2</td>
<td>13.8</td>
</tr>
<tr>
<td>Increase in absorbance</td>
<td>2.0</td>
<td>4.6</td>
<td></td>
</tr>
</tbody>
</table>

* Isolate equivalent to twice weight of beans used for other extracts in the table.

Figure 1. Absorbance of aliquots obtained by column chromatography on DEAE cellulose:
fractions obtained by bulking of aliquots.

The isolate prepared by the method of Sohonie and Ambe² was low in nitrogen
content. Since other trypsin inhibitor preparations had nitrogen contents of 13.0 to
16.7 %,¹² the purity of this preparation was probably low. The method of Garlich and
Nesheim¹⁰ yielded material with an activity half that expected from in vitro estimations
with the whole bean and with a nitrogen content approximately half that expected from
other data.¹²

The protein contents of aliquots obtained after column chromatography were plotted
against tube number. These results are shown in Figure 1. Fraction 1 contains the larger
protein peak. For estimation of t.i.a. the effluent was divided into seven fractions as shown in Figure 1. However, all fractions showed only slight t.i.a. and there was little difference between fractions as is shown in Table 5.

4. Discussion

This study demonstrates \textit{in vitro} evidence for the presence of t.i.a. in the sample of field bean meal used by Wilson and McNab\textsuperscript{1} in chick growth studies. Furthermore, t.i.a. was present in both the cotyledons and testa of the field bean. Dehulling of the bean is not, therefore, a means of eliminating t.i.a. Autoclaving at 110 °C for a period of 40 min eliminated t.i.a. Chick growth was increased on diets which contained high levels of bean meal when the beans were autoclaved at 120 °C for 30 min but further autoclaving produced no benefit.\textsuperscript{1} However, chemical detection of t.i.a. is not conclusive evidence of an adverse nutritional effect. Even when a growth response is obtained after autoclaving which coincides with a loss of inhibiting activity the bean may contain other heat-labile anti-nutritive factors which were not assayed but which may be responsible for the observed effects on growth.

\begin{table}
\centering
\caption{Absorbance ($\times 10^2$) of assay mixtures containing fractions 1 to 7 obtained by column chromatography}
\begin{tabular}{ll}
\hline
Trypsin concentration (mg/100 ml) & \\
0 & 100 \\
\hline
Absorbance of mixture + fraction 1 & 16.0 & 28.0 \\
Increase in absorbance & & 12.0 \\
Absorbance of mixture + fraction 2 & 8.3 & 22.5 \\
Increase in absorbance & & 14.2 \\
Absorbance of mixture + fraction 3 & 7.2 & 22.6 \\
Increase in absorbance & & 15.4 \\
Absorbance of mixture + fraction 4 & 7.2 & 22.7 \\
Increase in absorbance & & 15.5 \\
Absorbance of mixture + fraction 5 & 6.5 & 21.5 \\
Increase in absorbance & & 15.0 \\
Absorbance of mixture + fraction 6 & 15.5 & 28.7 \\
Increase in absorbance & & 13.2 \\
Absorbance of mixture + fraction 7 & 8.4 & 23.9 \\
Increase in absorbance & & 15.5 \\
Absorbance of blank mixture & 8.5 & 25.0 \\
Increase in absorbance & & 16.5 \\
\hline
\end{tabular}
\end{table}

References