On the Relationship between Free and Latent Beta-Amylases in Wheat[1]

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ABSTRACT

Free beta-amylase, extracted from flour with water or aqueous sodium chloride, has been compared with latent beta-amylase, liberated from glutenin with 1-thioglycerol. Both forms are similar on DEAE and CM-cellulose ion-exchange chromatography and polyacrylamide disc electrophoresis. Both forms contain two major components.

A large part of the beta-amylase of ungerminated wheat is known to occur in a latent and insoluble form. Rowsell and Goad (1) have shown that this beta-amylase is associated with the glutenin fraction. They further concluded that the beta-amylase was bound to the glutenin by disulfide linkages (2). The beta-amylase could be released by splitting the disulfide linkages with a chemical agent such as sodium sulfite or various thiols or by splitting peptide bonds in the glutenin with a proteolytic enzyme. Although much is known concerning the free beta-amylase of wheat which is active and soluble in water or dilute salt solutions (3,4,5), very little

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is known about the latent beta-amylase[2]. Pollock and Pool (6) have found that in barley, latent beta-amylase, which had been liberated with thioglycollic acid, resembled the free beta-amylase.

The present communication describes the use of cellulosic ion-exchange chromatography to compare free beta-amylase, soluble in water or salt, with latent beta-amylase, released from glutenin with 1-thioglycerol.

MATERIALS

The wheat flour used was a commercial straight-grade sample and was untreated. It contained 13.7% protein (N X 5.7) and 0.46% ash on a 14% moisture basis. Malted hard red spring wheat was purchased from Canada Malting Co. Ltd. It contained 13.1% protein (N X 5.7) and 1.5% ash on a 13.5% moisture basis. 1-Thioglycerol was purchased from Aldrich Chemical Co. Microgranular N,N-diethylaminoethyl cellulose (DE-32) and carboxymethyl cellulose (CM-32) were purchased from Reeve Angel Co.

METHODS

Preparation of Glutenin

A dough was prepared by mixing 50 g. of flour with 55 ml. of cold water in the GRL mixer for 2.5 min. The dough ball was placed in a beaker of water and allowed to stand for 1 hr. The dough was then washed intensively in turn with cold tap water, 4.0% sodium chloride, and finally 70% ethanol. The resultant glutenin was finally washed thoroughly with cold distilled water to remove the ethanol. An aqueous extract of this glutenin contained no beta-amylase activity.

Extraction of Latent Wheat Beta-Amylase

To the glutenin prepared from 50 g. flour was added 30 ml. of 1.24M 1-thioglycerol. The suspension was stirred for 2 hr. at room temperature. All extracts were centrifuged at 4°C. at 12,000 r.p.m. (17,000 X g) for 20 min., and all the supernatant containing beta-amylase was retained.

Extraction of Free Wheat Beta-Amylase

Wheat flour (10 g.) was added to 20 ml. of 1% sodium chloride and the suspension stirred for 2 hr. at room temperature. In one experiment, water was used as extractant in place of 1% sodium chloride.

Extraction of Malted Wheat Beta-Amylase

Malted wheat was finely ground into a whole wheat flour with a Wiley mill (equipped with a 1-mm. mesh sieve) and 10 g. of this flour was extracted with 20 ml. of 1% sodium chloride at 4°C. for 2 hr. To inactivate alpha-amylase, the pH of the extract was lowered to 3.6 and held for 2 hr. at 4°C.

Ion-Exchange Chromatography

Ion-exchange chromatography at basic pH was carried out as described previously (7) using a 2 X 48-cm. column of microgranular N,N-diethylaminoethyl (DEAE) cellulose equilibrated with 0.2M Tris-HCl, pH 8.0 containing 0.001M

[2]The present communication will use the terminology of Pollock and Pool (6) in which the free beta-amylase refers to that portion of the enzyme soluble in water or salt solution, while the portion chemically bound and insoluble and released by reducing substances or proteolytic enzymes will be referred to as latent beta-amylase.

ethylenediaminetetraacetic acid (EDTA). To elute the proteins, a linear salt gradient was prepared from a two-chambered gradient device. The first chamber contained 800 g. of stirring 0.2M Tris-HCl, pH 8.0 containing 0.001M EDTA into which flowed the contents of the second chamber consisting of 800 g. of 0.2M Tris-HCl, pH 8.0 containing 0.001M EDTA and 0.21M sodium chloride. Protein was measured at 280 mμ with a Vanguard ultraviolet analyzer equipped with 0.66-cm. quartz cells. Ion-exchange chromatography at acidic pH was carried out on a 2.5 × 26-cm. column of carboxymethyl (CM) cellulose equilibrated with 0.03M sodium acetate, pH 4.6 containing 0.001M EDTA. Elution of protein was again carried out using a linear salt gradient prepared from a two-chambered gradient device. The first chamber contained 800 g. of stirring 0.03M sodium acetate, pH 4.6 containing 0.001M EDTA into which flowed the contents of the second chamber consisting of 800 g. of 0.03M sodium acetate, pH 4.6 containing 0.001M EDTA and 0.086M sodium chloride. Detection and collection of proteins were made with a LKB Recychrom system. Extracts to be subjected to ion-exchange chromatography were adjusted to the appropriate pH with dilute HCl or NaOH and then dialyzed against the starting buffer.

Beta-Amylase Activity

Beta-amylase activity was measured by the colorimetric method of Bernfeld using 3,5-dinitrosalicylic acid (8). The substrate used was soluble starch Merck (according to Lintner). A unit of beta-amylase activity was expressed in terms of the amount of enzyme necessary to liberate 1 mg. of maltose in 5 min. at 20°C. from 1% soluble starch at pH 4.6. The hydrolysis of substrate was not allowed to proceed beyond 15%.

Detection of Alpha-Amylase

Alpha-amylase, if present in the chromatographic effluent, would interfere with the determination of beta-amylase by the above method. Its absence was indicated by the following test: 1 ml. of 1% soluble starch was added to 3 ml. of enzyme solution and incubated at 25°C. for 48 hr. Addition of a solution containing 0.01% iodine and 1% potassium iodide gave a blue coloration indicating that starch degradation was not complete as was found if a combination of alpha- and beta-amylase were present.

Polyacrylamide Disc Electrophoresis

This was carried out as described previously (7). Starch was incorporated into the gels as before in order to detect regions of beta-amylase activity.

RESULTS AND DISCUSSION

Solubilization of the latent beta-amylase was achieved by treating glutenin with 1-thioglycerol. Solubilization with proteolytic enzymes was not used as the latent beta-amylase would be released with fragments of glutenin attached to the enzyme. This might give irreproducible chromatography.

Ion-exchange chromatography of beta-amylase extracts of flour was first carried out on a 2 × 48-cm. column of DEAE cellulose equilibrated with 0.2M Tris-HCl, pH 8.0 containing 0.001M EDTA. The proteins were eluted from the column with a salt gradient, increasing linearly in concentration to 0.21M. Chromatograms illustrating the protein and enzyme elution curves of the free beta-amylases from

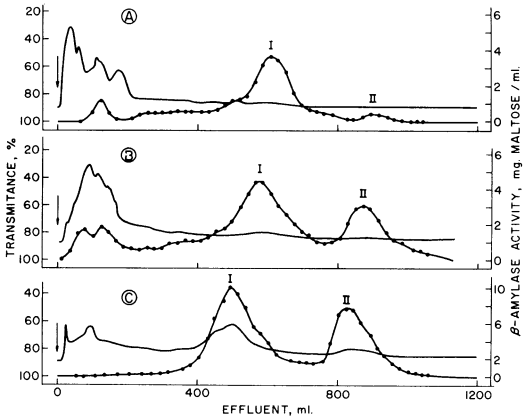

Fig. 1. Chromatography on DEAE cellulose of A, water-soluble beta-amylase extract, B, salt-soluble beta-amylase extract, C, latent beta-amylase extract. Solid circles, beta-amylase activity; solid line, percent transmittance; arrow indicates start of gradient.

water and salt extracts and from an extract of latent beta-amylase are shown in Fig. 1. Comparison of Fig. 1, A and B indicates that water-soluble and salt-soluble free beta-amylases differ in the proportion of component II present. This component could presumably be more globulin-like in character. Comparison of Fig. 1, B and C indicates that the salt-soluble free beta-amylase and latent beta-amylase have two major components with comparable elution profiles. The salt-soluble free beta-amylase has in addition one or more minor components which come off very early in the gradient.

To ascertain whether the apparent similarity of the free and latent beta-amylases was consistent under other ion-exchange conditions, chromatography at acidic pH was carried out. A 2.5 × 26-cm. column of CM cellulose equilibrated with 0.03M sodium acetate containing 0.001M EDTA was used and the proteins were eluted with a salt gradient increasing linearly in concentration to 0.086M. The resulting chromatograms illustrating the protein and enzyme elution curves of salt-soluble free and latent beta-amylase extracts on CM cellulose at pH 4.6 are shown in Fig. 2, A and B. Comparison of the enzyme elution curves of the two chromatograms indicates again that the free and latent beta-amylase extracts have two components with similar elution behavior. The free beta-amylase extract contains, in addition, at least two minor components. Components I from the free and latent beta-amylases were combined and rechromatographed under the same elution conditions. As shown in Fig. 2, C, one major peak resulted which eluted at the same position as the previous individual components. A similar result was obtained on rechromatographing an extract in which both the free and latent beta-amylase components II were joined. This is shown in Fig. 2, D.

The findings here on the close similarity in chromatographic behavior of free and latent beta-amylases strongly suggest that they are essentially identical. Two major forms appear to be present in both cases. A similar situation is believed to exist in barley. For example, Pollock and Pool (6) have found evidence using zonal electrophoresis that free and latent barley beta-amylases were very similar, and LaBerge et al. (9) found that barley extracted with buffer containing 1-thioglycerol

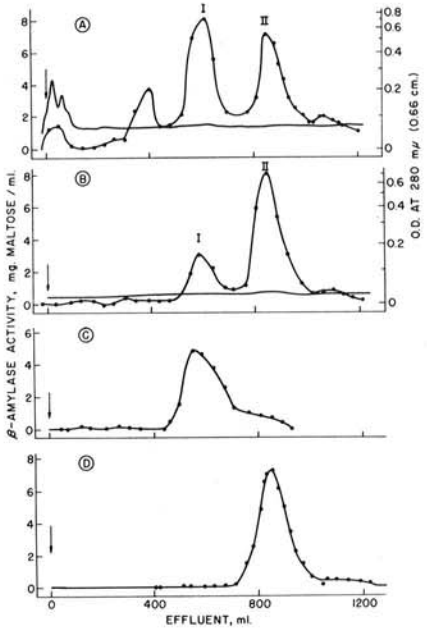

Fig. 2. Chromatography on CM cellulose of A, salt-soluble free beta-amylase extract, B, latent beta-amylase extract, C, components I from free and latent beta-amylase extracts rechromatographed, D, components II from free and latent beta-amylase extracts rechromatographed. Solid circles, beta-amylase activity; solid line, absorbance; arrow indicates start of gradient.

consisted of 2 beta-amylases. It should be noted that while the proportions of beta-amylase components in barley relative to each other were found to be thiol-sensitive, such is not the case for the beta-amylase system of wheat.

Polyacrylamide disc electrophoresis was carried out on both the free and latent beta-amylases obtained from ion-exchange chromatography on CM cellulose. Starch containing gels to detect beta-amylase activity indicated that components I and II of Fig. 2, A and B could not be resolved, although preparations in which both were present gave a more diffuse band. Combinations of free and latent beta-amylases gave a single band indicating that they were electrophoretically similar. Protein staining of disc gels indicated that one protein was present in preparations of latent beta-amylase I (Fig. 2, B) which corresponded to the region of amylase activity. This is shown in Fig. 3. Preparations of latent beta-amylase II (Fig. 2, B) contained

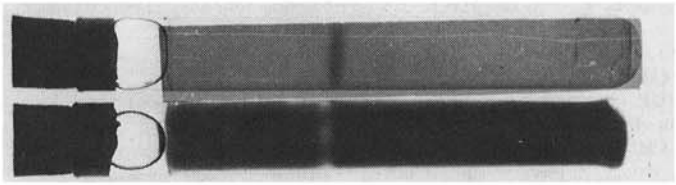

Fig. 3. Disc electrophoresis of bound beta-amylase component I from CM cellulose chromatography. Bottom disc, stained for beta-amylase activity; top disc, stained for protein.

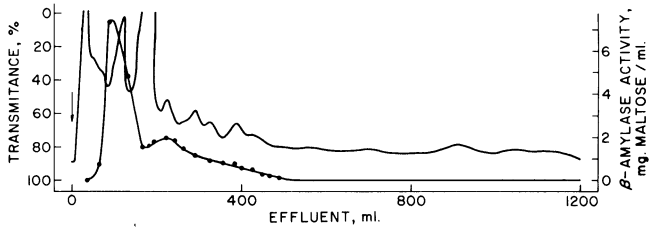

Fig. 4. Chromatography on DEAE cellulose of a salt-soluble beta-amylase extract from malted wheat. Solid circles, beta-amylase activity; solid line, percent transmittance; arrow indicates start of gradient.

at least three proteins although extracts which had sat for 2 days or more contained one major protein which corresponded to the region of amylase activity. Ion-exchange chromatography on CM-32 under the conditions described is therefore a suitable method for purifying latent beta-amylase.

During germination of wheat, the amount of free beta-amylase increases greatly while the amount of latent enzyme decreases. In an additional experiment designed to determine if part or all of the increase in free enzyme arises from the latent enzyme possibly via breakage of disulfide bonds, the ion-exchange chromatography of a malted wheat extract was carried out. Figure 4 shows the ion-exchange chromatography on DEAE cellulose at pH 8.0. Comparison with Fig. 1 indicates that the beta-amylase has a very different chromatographic profile and is eluted with much less salt. A control experiment with ground whole wheat flour from a hard red spring sound wheat under the same conditions gave a chromatographic pattern similar to Fig. 1, B indicating that the acid treatment did not cause the difference. These findings suggest that the increase in free beta-amylase is not due to release of latent beta-amylase by scission of disulfide bonds. The possibility is not ruled out, however, that the latent beta-amylase is released from the glutenin by proteolytic enzymes. This could indeed be possible in view of the large increase in proteolytic activity during germination.

Acknowledgment

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