PROTEIN α-AMYLASE INHIBITORS FROM WHEAT FLOUR

W. PACE, R. PARLAMENTI, A. UR RAB, V. SILANO, and L. VITTOZZI, Laboratorio di Tossicologia, Istituto Superiore di Sanita, Rome

Cereal Chem. 55(2): 244-254

ABSTRACT

A number of pure gliadin fractions were tested for inhibitory activity on human salivary, yellow mealworm, and pig pancreatic α-amylases; all were inactive. Many extraction solvent systems commonly considered typical of wheat gliadins were found to be highly effective for extraction of albumin α-amylase inhibitors from wheat flour and gluten. Extraction of the bulk of amylase inhibitors from wheat flour was achieved with five consecutive treatments with 0.15*M* NaCl; use of 70% aqueous ethanol instead of the salt solution did not improve extraction of residual α-amylase inhibitors. These data all indicate that gliadin amylase inhibitors in wheat flour account for only a small fraction of the total inhibitor content. α-Amylase inhibitors were tested at different stages of wheat kernel development. Inhibitory activity steadily increased during seed maturation and reached its maximum at the maturity stage. Throughout seed maturation, amylase inhibitory activity was inversely related to wheat α-amylase activity, but at no stage of kernel development did amylase inhibitors extracted from mature kernel inhibit wheat α-amylase. After germination, the content of amylase inhibitors of the kernel rapidly decreased and no inhibitory activity was detectable in newly formed tissues such as roots and coleoptiles. Preliminary comparison of gel filtration patterns of amylase inhibitors extracted from the kernel of 11 nullitetrasomic wheat strains showed a complex genetic control of α-amylase inhibitors.

Wheat kernel contains a number of albumin components that actively inhibit many α-amylases from sources other than wheat but that are inactive with α-amylase in wheat (1). Buonocore *et al.* (1) have extensively characterized several albumin α-amylase inhibitors and thoroughly investigated their interactions with the inhibited amylases. In 1973, Strumeyer and Fisher (2) preliminarily described the only α-amylase inhibitor in the gliadin fraction of wheat kernel. They extracted an α-amylase protein inhibitor from a commercial preparation of wheat gliadin that showed two major bands corresponding to the α-gliadins on electrophoresis.

To our knowledge, reports of extensive work on further characterization studies of this inhibitor have not been published. Others (3–6) have presented evidence that amylase inhibitors in the gliadin fraction are present in wheat flour, commercial wheat gliadin, or gluten. This is based mainly on their apparent solubility in solvents commonly considered typical for gliadins. The possibility that amylase inhibitory activity is common to both the albumin and gliadin fractions is significant enough to clarify structural features essential for inhibitory activity because of the large structural differences existing between these two protein groups. Moreover, the suggestion that α-gliadin actually is an amylase inhibitor deserves further investigation because of the toxic role attributed to α-gliadin in celiac disease (7–9) and because this is the first biologic activity described for wheat gliadin.

This article deals with α-amylase inhibition tests done with a number of highly purified and crude gliadin preparations to confirm previous reports on gliadin

Address correspondence to: V. Silano, Laboratorio di Tossicologia, Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Roma, Italy.

0009-0352/78/000028$03.00/0
© 1978 The American Association of Cereal Chemists

amylase inhibitors in wheat. Moreover, to clarify the significance of these naturally occurring inhibitors, we have quantitatively studied the production of wheat α-amylase and α-amylase inhibitors at different stages of kernel development and assayed amylase inhibitors in different tissues of the wheat plant. We have also extended previous experiments showing no inhibition of wheat α-amylase by albumin inhibitors (10,11) as well as those suggesting that genes coded for wheat α-amylase inhibitors are mainly located on chromosomes 3B and 3D (12,13).

MATERIALS AND METHODS

Pure Gliadin Fractions

Dr. F. R. Huebner supplied gliadin fractions coded as γ_1, γ_2, γ_3, β_5, β_6, α_{7-8}, α_{10}, and α_{12}. Dr. D. D. Kasarda supplied the A-gliadin.

α-Amylase Inhibitors From Wheat Flour and Gluten

The flour used for the experiments reported here was a 70% extraction white flour (ash less than 0.5%) obtained from milling a *Triticum aestivum* wheat (variety Mentana). For the extraction of inhibitors, the solvent systems listed in Table I were used. The extraction and purification procedures are shown in Fig. 1. In all cases, 100 g of flour were extracted with 1.5 L of the solvent system chosen four consecutive times by resuspending the residue of the previous extraction in fresh solvent each time. Each extraction was done for 1 hr under mechanical stirring at room temperature; centrifugations lasted 20 min at 40,000 $\times g$. Unless otherwise stated, all fractions obtained were dialyzed against water and freeze-dried. The extraction with NaOH (pH 9) was done according to Saunders (14); purification of this extract included neutralization with 1*M* HCl,

TABLE I
Extraction of α-Amylase Inhibitors From Wheat Flour With Different Solvent Systems

Fraction No.	Solvent System	pH	Purification Procedure	α-Amylase Inhibitor Yield[a] (%)
1	Distilled water	6.2	Salting out at 0.4*M* $(NH_4)_2SO_4$	Trace
2	Distilled water	6.2	Salting out 0.4–4.0*M* $(NH_4)_2SO_4$	19
3	Dilute HCl	3.0	...	26
4	Dilute Na_2CO_3	9.0	...	45
5	Dilute NaOH	9.0	Precipitation with acetone	27
6	0.26*M* $(NH_4)_2SO_4$, HCl	3.0	Salting out at 4.0*M* $(NH_4)_2SO_4$	73
7	70% Ethanol, HCl	3.0	...	100
8	70% Ethanol	6.2	...	17
9	70% Ethanol	6.2	Precipitation at −20°C	8
10	70% Ethanol	6.2	Precipitation with ethanol	6
11	70% Ethanol	6.2	Precipitation with acetone	11

[a]Amylase inhibitor yield is expressed as percent of the inhibitor amount extracted with 70% ethanol (pH 3.0) taken as 100%.

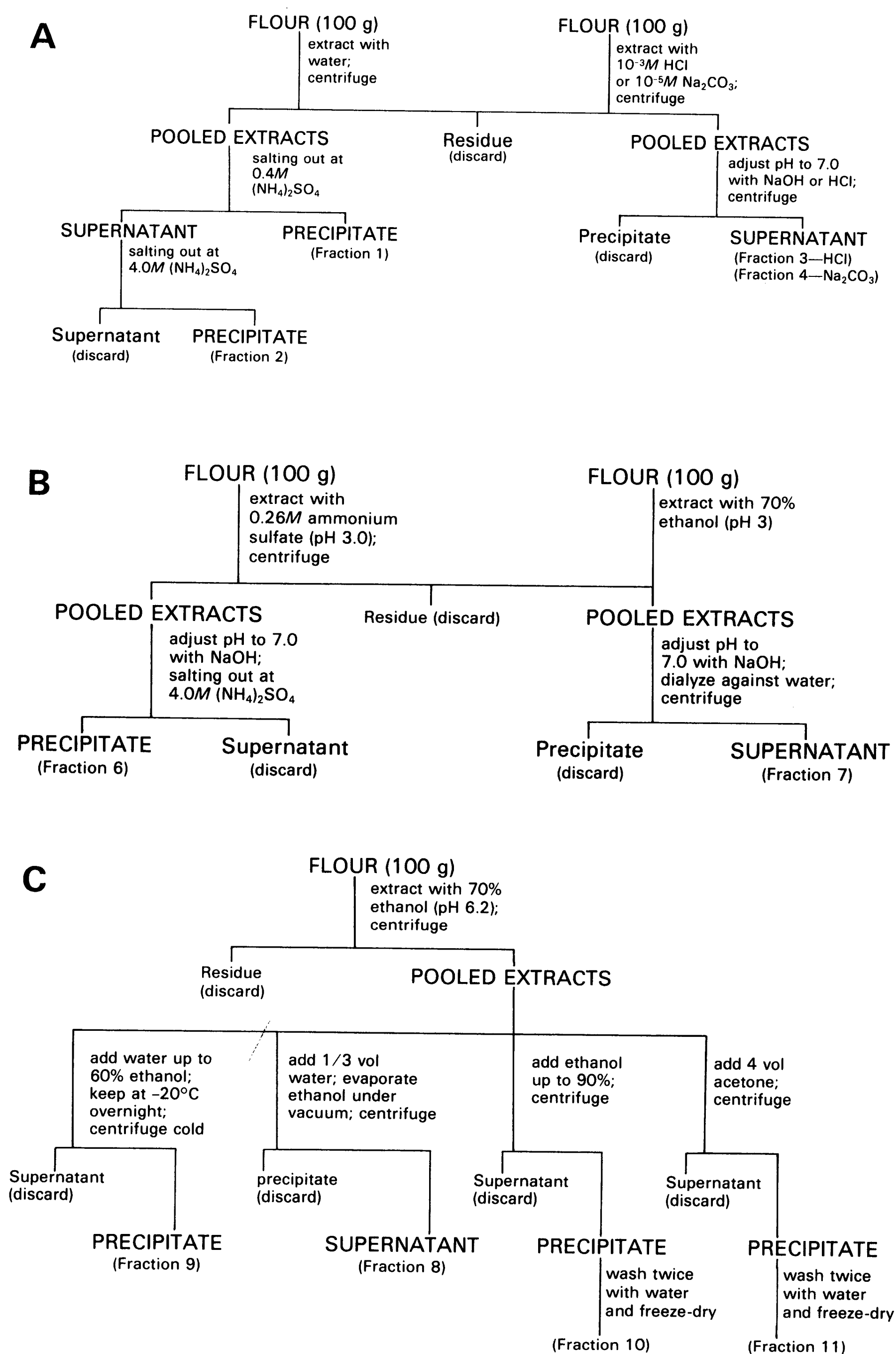

Fig. 1a, b, c. Procedures for extraction and purification of α-amylase inhibitors from wheat flour with different solvent systems.

heating at 80°C, and precipitation with acetone. This fraction was coded as Fraction 5.

To achieve exhaustive extraction of amylase inhibitors, 70% extraction flour (100 g) as well as whole wheat flour (100 g) were extracted five consecutive times at room temperature for 3 hr in a shaker with 150 ml of 0.15*M* NaCl. The extraction residue was then extracted three consecutive times with 150 ml of a water-ethanol (30:70, v/v) solution under identical experimental conditions. All extracts obtained were individually submitted to amylase inhibitor assay with no further treatment.

α-Amylase inhibitors were extracted from commercial gluten (Buitoni, Perugia, Italy) following the procedure of Schmidt and Puls (5). One hundred grams of gluten were suspended in 500 ml of methanol under magnetic stirring; 0.5*N* HCl was added to lower pH to 3.5. After 45 min of mixing, the suspension was neutralized with 0.5*N* NH_4OH and centrifuged for 10 min at 40,000 × *g* at 25°C. The clear supernatant was kept at −10°C overnight to allow inhibitor precipitation. The precipitate was collected by centrifugation at −5°C and freeze-dried.

The inhibitory activity of all these preparations was determined on human salivary α-amylase by using the dinitrosalicylate reagent according to the procedure that Silano *et al.* (15) described; the amylase was freeze-dried crude saliva. One inhibition unit (IU) is the amount of inhibitor that causes 30% inhibition of the amylase under the described conditions. The amount of α-amylase used was that which would produce 1 mg of maltose in 5 min under our experimental conditions. The amylase inhibitor preparations were submitted to anodic (pH 8.5) and cathodic (pH 4.3) polyacrylamide gel electrophoresis as Deponte *et al.* (16) described.

α-Amylase Inhibitors and α-Amylase Activity From Maturing Kernel and Other Plant Tissues

The soft winter wheat (variety Mara) used in these studies was sampled at the times after heading indicated in Fig. 2. Intact heads of wheat plants were excised and stored in deep freeze until analysis. Wheat germination was done under the conditions that Kruger (17) described; roots, coleoptiles, and their attachments to germinating seeds were excised and stored with the remaining grain under identical conditions. The extraction of amylase and amylase inhibitors from the tissues studied was done on 0.1 g of finely ground tissue with 18m*M* $CaCl_2$ according to the procedure that Perten (18) described. α-Amylase activity of the extracts was determined in an acetate buffer (pH 4.7) at 30°C with limit dextrin as substrate using the method of the International Association for Cereal Chemistry and expressed as amylase units (AU) as Perten (18) described. Inhibitory activity of the extracts on *Tenebrio molitor* L. α-amylase was determined with the I_2/KI reagent according to the procedure that Silano *et al.* (11) described. This was done after thermal inactivation of endogenous amylase activity that was achieved by heating the extracts from the kernel at 90°C for 30 min and those from other tissues at 80°C for 10 min. As such, thermal treatments reduced the activity of the purified 0.19 inhibitor of Silano *et al.* (15) of about 75 and 25%, respectively, experimental values of inhibitory activities of the extracts from kernel and other tissues were correspondingly increased, assuming that equal losses of activity would occur for the amylase inhibitors present in crude extracts. With this method, 1 IU is the amount of inhibitor that causes 30%

inhibition of the amylase under the described conditions. The amount of α-amylase used was that which would produce 50% hydrolysis of the added starch.

α-Amylase Inhibitors From Nullitetrasomic Wheats

α-Amylase inhibitors were extracted with distilled water on 13–20 seeds of each line tested according to Bedetti *et al.* (19) after removal of embryos. Water extracts were submitted to gel filtration on a Sephadex G-100 column as previously described (19), and apparent molecular weights of the eluted inhibitor fractions calculated from the corresponding retention volumes according to Andrews (20). Inhibitory activity of the column eluate on human salivary and *Tenebrio molitor* L. (yellow mealworm) α-amylase was determined continuously as previously described (21). With this method, 1 IU represents the inhibitor amount that gives 30% inhibition of the amylase under the described conditions. The amount of α-amylase used was that which would produce 0.3 μeq of maltose under the described experimental conditions (21). Dr. A. Bozzini supplied the nullitetrasomic strains tested.

RESULTS

Pure Gliadin Fractions

A number of highly purified gliadins, including the γ_1-, γ_2-, γ_3-, β_5-, β_6-, α_7-, α_{7-8}-, and α_{10}-gliadin fractions of Huebner and Rothfus (22) and the A-gliadin fraction of Kasarda *et al.* (23) were tested for inhibitory activity of human salivary, yellow mealworm midgut, and pig pancreatic α-amylases by using the

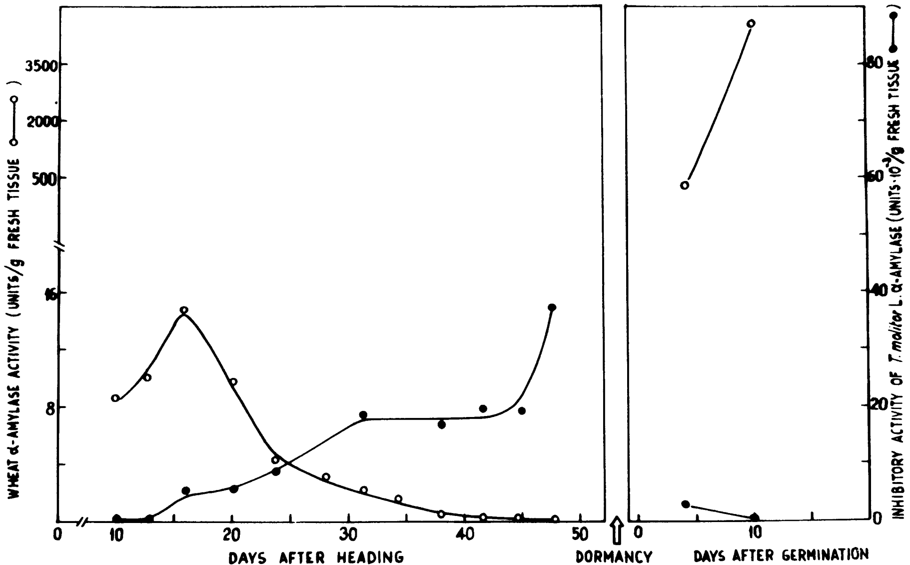

Fig. 2. Assay of α-amylase inhibitors (•) and α-amylase activity (o) from wheat kernel at different stages of development.

dinitrosalicylate reagent according to the procedure that Silano *et al.* (15) described. All the gliadins were found inactive, even when tested at concentrations 100 times higher than those needed to observe inhibition with wheat albumins (1). Only the α_{12}- gliadin fraction of Huebner and Rothfus (22) showed a slight inhibitory activity corresponding to about 1/100 of that exhibited by some wheat albumin fractions (1). This gliadin fraction, however, was the only one that showed impurities on gel electrophoresis, with electrophoretic mobilities and staining properties with aniline blue-black typical of wheat albumins (24). The possibility that any α-amylase inhibitors present in the gliadin fractions tested had been inactivated during the purification procedure, which included (22) treatments in buffers containing 0.015*M* HCl, 0.03*M* acetic acid, and 2*M* dimethylformamide, was explored by submitting the purified 0.19 albumin inhibitor of Silano *et al.* (15) to a 48-hr incubation in a buffer containing the three above-mentioned chemicals. Under such conditions, no inactivation of the 0.19 inhibitor was observed.

Extraction of α-Amylase Inhibitors With Different Solvents

An acidic (pH 3) water-ethanol solution (30:70, v/v) was the most effective solvent for α-amylase inhibitor extraction. Amylase inhibitor yield with this solvent was approximately $0.7 \cdot 10^6$ IU/100 g of flour. Relative amounts of amylase inhibitors extracted from wheat flour with different solvent systems as compared with the inhibitor amount extracted with acidic 70% ethanol taken as 100% are shown in Table I. At pH 6.2, almost equal inhibitor yields were obtained with water and 70% ethanol (Fractions 2 and 8); at pH 3.0, a 0.26*M* ammonium sulfate solution (Fraction 6) was a much more effective extractant than was water (Fraction 3). The extraction done with Na_2CO_3 (pH 9) (Fraction 4) gave an inhibitor yield intermediate between those observed at pH 3.0 and 6.2, whereas that performed with NaOH, according to Saunders (14) (Fraction 5), was not particularly effective as compared with the other extraction procedures. Almost all of the inhibitory activity extracted with water at pH 6.2 was salted out with ammonium sulfate in the range 0.4–4.0*M* (Fractions 1 and 2), thus showing that no significant α-amylase inhibitor activity is associated with the gliadin-like protein fraction from flour. The purification of amylase inhibitors extracted with 70% ethanol (pH 6.2) was attempted with several methods, including precipitation at low temperature or with organic solvents according to Schmidt and Puls (5) (Fractions 9–11), but in all cases, low inhibitor yields were obtained. We also extracted α-amylase inhibitors from a commercial sample of gluten by following the procedure of Schmidt and Puls (5). About 500,000 IU were obtained from 100 g of gluten. When one considers that 100 g of gluten usually derive from 1.5 kg of flour, the inhibitor content of gluten appears to be about 1/20 that extracted from wheat flour with 70% ethanol.

Inhibitory specific activities of all these extracts were in the range 0.4–1.0 IU/μg of protein. Taking into account the large errors affecting determination of both protein and amylase inhibitor contents in crude preparations, one apparently cannot consider the differences observed in such a range significant. When submitted to polyacrylamide gel electrophoresis at both acidic and alkaline pH, the inhibitor fractions of Table I and the extract from wheat gluten exhibited, with the main exception of Fractions 1 and 2, similar qualitative patterns showing bands with mobilities and staining properties with aniline blue-

black typical of both wheat albumins and gliadins (24). Fractions 1 and 2 exhibited patterns typical of wheat gliadins or albumins, respectively (24).

Exhaustive extraction of amylase inhibitors from both 70% extraction flour and whole wheat flour was attempted with consecutive treatments of flour with 0.15*M* NaCl (Fig. 3). After five treatments of flour with 0.15*M* NaCl, a 70% ethanol solution was used to evaluate whether any quantitatively significant inhibitor fraction could be specifically extracted by the alcoholic solution. As shown in Fig. 3, the results obtained provide no support to the presence of such a fraction in the extraction flour or whole flour.

α-Amylase Activity and α-Amylase Inhibitors at Different Stages of Plant Development

As shown in Fig. 2, α-amylase activity rapidly increased on fertilization and reached the maximum value about 16 days after heading; then the amylase activity rapidly decreased and reached the minimum value at the maturation stage. A tremendous increase in the enzyme activity was observed after germination of the kernel. Stability of the enzyme strongly varied during maturation. α-Amylase activity extracted from seeds at ten days after heading was not affected even after 5 hr at 60° C, whereas the amylase activity from mature seeds was destroyed after 4 hr at 25° C. These differences might possibly be related to other differences (25,26). Ten days after germination, coleoptiles contained 3.9 AU/g and roots 9.0 AU/g of fresh tissue.

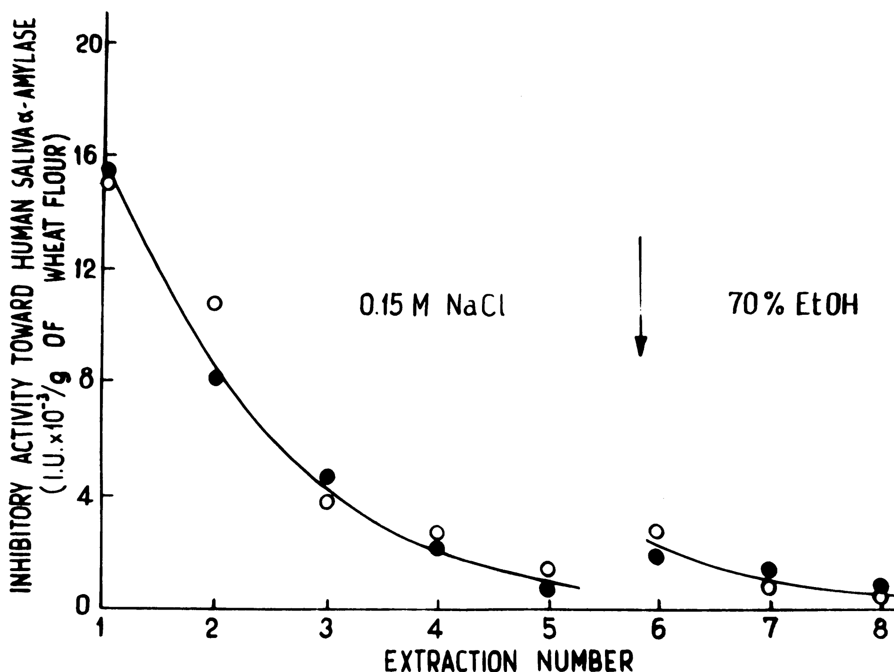

Fig. 3. Consecutive extractions of α-amylase inhibitors from 70% extraction flour (o) and whole wheat flour (•) with 0.15*M* NaCl and 70% ethanol.

Inhibitory activity on yellow mealworm amylase steadily increased during seed maturation and reached its maximum value at the maturity stage. These findings are basically in agreement with the qualitative results that Sandstedt and Beckord (27) reported on inhibitory activity on human salivary α-amylase. Moreover, on germination, inhibitory activity on the insect amylase rapidly decreased (Fig. 2) and no α-amylase inhibitory activity was detectable in newly formed tissues such as coleoptiles and roots.

α-Amylase activity of maturing kernels sampled at the times indicated in Fig. 2 as well as amylases extracted from coleoptiles and roots were tested in the presence of the albumin fractions (60,000, 24,000, and 12,000 mol wt) that were extracted from mature kernel according to the procedure that Silano *et al.* (11) described. We could not detect any inhibitory activity of these albumin fractions toward any amylase tested, even when wheat albumins were present at a concentration as high as 100 μg/ml.

α-Amylase Inhibitors From Nullitetrasomic Wheats

Eleven wheat strains, each one nullisomic for one chromosome and tetrasomic for a homologous chromosome, were analyzed for yellow mealworm and human salivary amylase inhibitors, basically following the procedure of Bedetti *et al.* (19). The procedure included fractionation of the inhibitors on a Sephadex G-100 column and automated continuous assay of the inhibitors in the column eluate. Although incomplete, such a screening clearly showed a complex genetic control of α-amylase inhibitors from wheat kernel (Fig. 4). From a qualitative standpoint, no differences were found among all the wheat strains tested. Three fractions (11,000, 22,000, and 60,000 mol wt) active on the insect amylase were present in all the wheat samples tested, including two samples of hexaploid Chinese spring and one tetraploid wheat (var. Aziziah). Two of these fractions (22,000 and 60,000 mol wt) were also active on the mammalian amylase. These results agree with those previously obtained with a number of tetraploid and hexaploid wheat cultivars (19). From a quantitative standpoint, an extremely low content of the 11,000 inhibitor fraction was observed in the nulli 6D-tetra 6B strain and high content of the 60,000 inhibitor fraction was observed in the nulli 5D-tetra 5B strain. Moreover, as compared with the two samples of Chinese spring wheat that were tested, significantly larger amounts of all three inhibitor fractions were present in the nulli 3B-tetra 3D strain. Finally, the content of the 11,000 inhibitor fraction was higher also in the nulli 3D-tetra 3B and nulli 3B-tetra 3A strains. Other quantitative differences as related to the contents of the inhibitor fractions are also shown in Fig. 4. Such differences are less significant, however, from a quantitative standpoint and are of doubtful importance.

DISCUSSION

In spite of the fact that Strumeyer and Fisher (2,3) have described an α-amylase inhibitor in the gliadin fraction of wheat flour, we have not been able to detect any inhibitory activity in human salivary, yellow mealworm, and pig pancreatic α-amylases in a number of purified gliadin fractions. Moreover, we have shown that α-amylase inhibitors in the albumin fraction can be extracted from wheat flour or commercial wheat gluten with solvents that are considered typical for wheat gliadins (*e.g.*, 70% ethanol, dilute acidic solutions). Our results

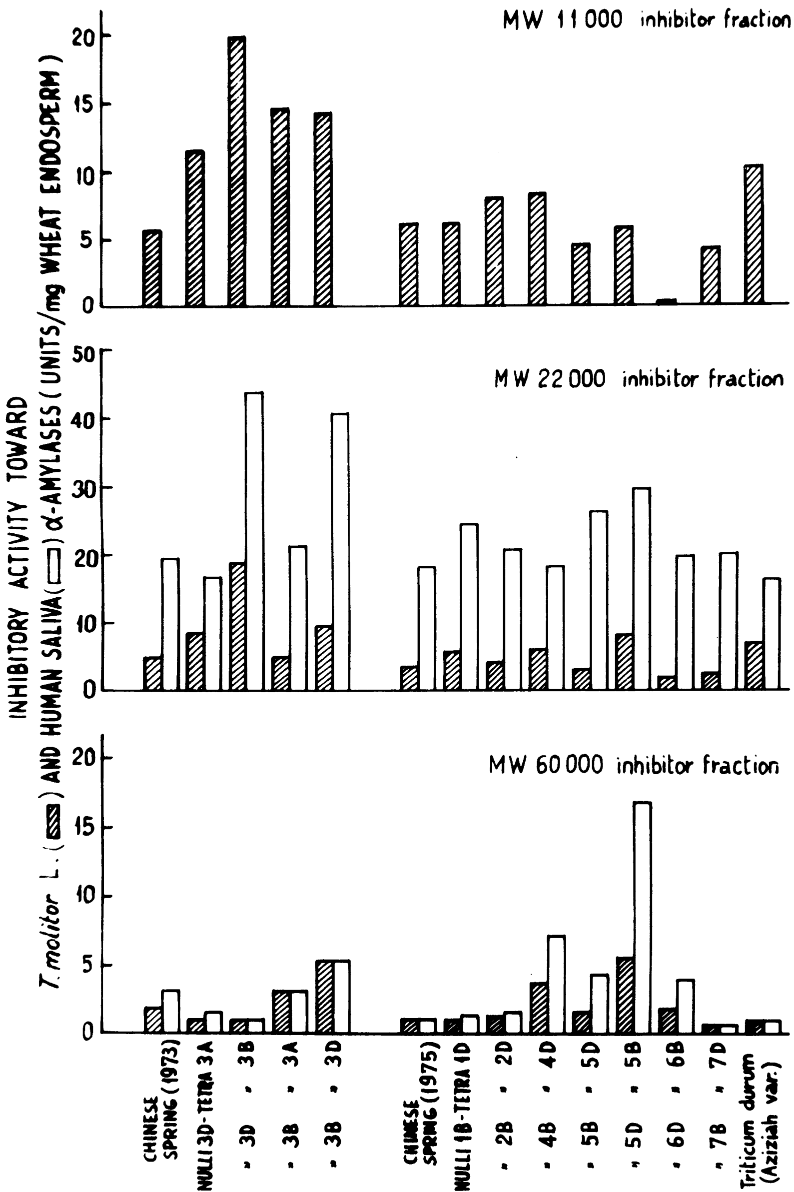

Fig. 4. Assay of α-amylase inhibitor fractions with different molecular weights from kernels of nullisomic-tetrasomic wheat strains.

suggest that if gliadin amylase inhibitors are present in wheat kernel, they account for a small amount of the total inhibitor content. As purified albumin inhibitors act at concentrations as low as 0.01–0.3 μg/ml, the possible presence of active albumin impurities, which are hardly detectable with the usual homogeneity tests, should be taken into account when isolating gliadin amylase inhibitors. At the moment, we have no satisfying explanation for the extraction behavior of α-amylase inhibitors that apparently are more effectively extracted at acidic pH both in the presence of 70% water-ethanol solution and 0.26*M* ammonium sulfate.

In 1946, Kneen and Sandstedt (28) reported that most of the inhibitor is located in the endosperm. This observation has been confirmed with microscopic studies (27) and with assay of inhibitor contents in different milling fractions (14). The linear relationship observed between inhibitor and starch contents of different milling fractions indicates that the inhibitors are closely associated with starch and are endospermic in nature. We have confirmed such findings, showing that α-amylase inhibitors are not present in coleoptiles and roots from germinated seeds. The inhibitor production in the kernel starts approximately eight days after heading and rapidly increases with maturation up to a maximum reached at full maturity. The inhibitor content of the kernel rapidly decreases after germination. We have observed an inverse relationship between the biosynthesis of wheat α-amylase and α-amylase inhibitors at different stages of kernel development, but at no stage under our experimental conditions could we detect any inhibition of wheat α-amylase by wheat albumins from mature kernel.

Others (12,13) have studied the location of genes controlling the synthesis of several wheat albumins, including some α-amylase inhibitors; they have suggested that homologous chromosome group 3 mainly controls albumins. We have studied the α-amylase inhibitory activity associated with fractions with different molecular weights extracted with water from a number of nullitetrasomic strains of wheat. The results obtained with this method of investigation indicate a complex genetic control of α-amylase inhibitors from wheat kernel, and at the moment they appear not to be easily comparable to those obtained from other authors.

Acknowledgments

We would like to thank Dr. F. R. Huebner for generously supplying the γ-, β-, and α-gliadin fractions, Dr. D. D. Kasarda for the A-gliadin sample, and Dr. A. Bozzini for the nullitetrasomic wheat strains.

Literature Cited

1. BUONOCORE, V., PETRUCCI, T., and SILANO, V. Wheat protein inhibitors of α-amylase. Phytochemistry 16: 811 (1977).
2. STRUMEYER, D. H., and FISHER, B. R. Purification and characterization of an amylase inhibitor from wheat gliadin. Fed. Proc. 32: 624 (1973).
3. STRUMEYER, D. H. Protein amylase inhibitors in the gliadin fraction of wheat and rye flour: Possible factors in coeliac disease. Nutr. Rep. Intern. 5: 45 (1972).
4. PULS, W., and KEUP, U. Influence of an α-amylase inhibitor on blood glucose, serum insulin and NEFA in starch loading tests in rats, dogs, and man. Diabetologia 9: 97 (1973).
5. SCHMIDT, D., and PULS, W. Amylase inhibitors. German. Offen. 2.028.739 (1970).
6. SCHMIDT, D., and PULS, W. Amylase inhibitors. German. Offen. 2.003.934 (1971).
7. HEKKENS, W. TH. J. M., HAEX, A. J., and WILLIGHAGEN, R. G. J. Some aspects of

gliadin fractionation and testing by a histochemical method. In: BOOTH, C. C., and DOWLING, R. H. (eds.). Coeliac disease. p. 11. Churchill Livingstone: Edinburgh (1970).
8. HEKKENS, W. TH. J. M., VAN DER AARSEN, C. J., GILLIAMS, J. P., LEMS-VAN KAN, PH., and BOUMAFROLICH, G. α-Gliadin structure and degradation. In: HEKKENS, W. Th. J. M., and PENA, A. S. (eds.). Coeliac disease. p. 39. Stenfert Kroese: Leiden (1974).
9. KENDALL, M. J., SCHNEIDER, R., COX, D. S., and HAWKINS, C. F. Gluten subfractions in coeliac disease. Lancet 2: 1065 (1972).
10. SHAINKIN, R., and BIRK, Y. α-Amylase inhibitors from wheat: Isolation and characterization. Biochim. Biophys. Acta 221: 502 (1970).
11. SILANO, V., FURIA, M., GIANFREDA, L., MACRI', A., PALESCANDOLO, R., RAB, A., SCARDI, V., STELLA, E., and VALFRE', F. Inhibition of amylases from different origins by albumins from the wheat kernel. Biochim. Biophys. Acta 391: 170 (1975).
12. CUBADDA, R. Chromosomal location of genes controlling the synthesis of some soluble proteins in *T. durum* and *T. aestivum*. In: SCARASCIA MUGNOZZA, G. T. (ed.). Genetics and breeding of durum wheat. p. 653. Biblioteca della Facolta di Agraria: Bari (1973).
13. ARAGONCILLO, C., RODRIGUEZ LOPERENA, M. A., CORBONERS, P., and GARCIA-OLMEDA, F. Chromosomal control of non-gliadin proteins from the 70% ethanol extract of wheat endosperm. Theoret. Appl. Genet. 45: 322 (1975).
14. SAUNDERS, R. M. α-Amylase inhibitors in wheat and other cereals. Cereal Foods World 20: 282 (1975).
15. SILANO, V., POCCHIARI, F., and KASARDA, D. D. Physical characterization of α-amylase inhibitors from wheat. Biochim. Biophys. Acta 317: 139 (1973).
16. DEPONTE, R., PARLAMENTI, R., PETRUCCI, T., SILANO, V., and TOMASI, M. Albumin α-amylase inhibitor families from wheat flour. Cereal Chem. 53: 805 (1976).
17. KRUGER, J. E. Changes in the amylases of hard red spring wheat during germination. Cereal Chem. 49: 391 (1972).
18. PERTEN, H. A colorimetric method for the determination of α-amylase activity (ICC Method). Cereal Chem. 53: 336 (1966).
19. BEDETTI, C., BOZZINI, A., SILANO, V., and VITTOZZI, L. Amylase protein inhibitors and the role of *Aegilops* species in polyploid wheat speciation. Biochim. Biophys. Acta 362: 299 (1974).
20. ANDREWS, P. Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J. 91: 222 (1964).
21. VITTOZZI, L., MORISI, G., and SILANO, V. Continuous automated assay of α-amylase inhibitors. J. Sci. Food Agr. 27: 449 (1976).
22. HUEBNER, F. R., and ROTHFUS, J. A. Gliadin proteins from different varieties of wheats. Cereal Chem. 45: 242 (1968).
23. KASARDA, D. D., BERNARDIN, J. E., and GAFFIELD, W. Circular dichroism and optical rotary dispersion of α-gliadin. Biochemistry 7: 3950 (1968).
24. MINETTI, M., PETRUCCI, T., CATTANEO, S., POCCHIARI, F., and SILANO, V. Studies of the differential staining of wheat albumins, globulins, and gliadins in polyacrylamide gel by aniline blue black. Cereal Chem. 50: 198 (1973).
25. MEREDITH, P., and JENKINS, L. D. Amylases of developing wheat, barley, and oat grains. Cereal Chem. 50: 243 (1973).
26. MARCHYLO, B., KRUGER, J. E., and IRVINE, G. N. α-Amylases from immature hard red spring wheat. Purification and some chemical and physical properties. Cereal Chem. 53: 157 (1976).
27. SANDSTEDT, R. M., and BECKORD, O. C. Photomicrographic studies of wheat starch amylolytic enzymes and the amylase inhibitor of the developing wheat kernel. Cereal Chem. 23: 548 (1946).
28. KNEEN, E., and SANDSTEDT, R. M. Distribution and general properties of an amylase inhibitor in cereals. Arch. Biochem. 9: 235 (1946).

[Received April 12, 1977. Accepted October 13, 1977]