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IDENTIFICATION OF AN ESSENTIAL TRYPTOPHANYL RESIDUE IN THE PRIMARY STRUCTURE OF GLUCOAMYLASE G2 FROM ASPERGILLUS NIGER

by

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Enzymatically active, N-bromosuccinimide oxidized glucoamylase G2 (EC 3.2.1.3) was prepared in the presence of the inhibitor acarbose and inactive, oxidized G2 with capacity to bind substrate was prepared in the presence of maltose. Four out of 15 tryptophanyl residues were oxidized in the first derivative and five in the latter. In order to identify this fifth and essential residue, tryptic fragments of the two G2 derivatives were isolated. The peptide fragment from the inactive G2 derivative containing the additional oxindolealanine residue was located in HPLC chromatograms by UV-absorption measurements. Normal and second derivative UV-spectra, amino acid composition and NH_2-terminal sequence of the isolated fragment led to identification of Trp(120) as a residue essential for activity. A short homologous amino acid sequence precedes both this Trp(120) and the Trp(83) which is involved in substrate binding in Taka-amylase A (J. Biochem. 95, 697-702 (1984)).

1. INTRODUCTION

With several carbohydrases, X-ray crystallography, UV-difference spectroscopy, NMR and fluorescence spectroscopy show that tryptophanyl residues are involved in substrate binding (1-4, 12, 13, 15, 16, 22). Glucoamylase (EC 3.2.1.3) which catalyzes the release of D-glucose from the non-reducing ends of starch and related oligo- and polysaccharides, has a tryptophanyl residue in subsite 1 of the substrate binding region (11, 23). Recently, a second essential tryptophan was found in glucoamylase from Aspergillus niger and these two residues apparently play different roles in the action of the enzyme (9).

In the present study we have identified a tryptophanyl residue required to maintain the catalytic activity as Trp(120) in the primary structure of glucoamylase G2 from A. niger. G2 differs from G1 by lacking a COOH-terminal region of approx. 100 amino acid residues, but the two forms have essentially the same activity on soluble substrates (31). The amino acid sequence of the enzyme (5, 6, 30) near the

Abbreviations: AH = aminohexyl; G1 and G2 = the larger and the smaller of the two forms of glucoamylase from A. niger (31); HPLC = high pressure liquid chromatography; NBS = N-bromosuccinimide; TFA = trifluoroacetic acid; Tris = 2-amino-2(hydroxymethyl)-1,3-propandiol.

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essential residue resembles that near a tryptophanyl residue of Taka-amylase A, proposed to interact with substrate in the active site cleft of this α-amylase (20, 33).

2. MATERIALS

A commercial preparation of Aspergillus niger glucoamylase (AMG 200L) was generously supplied by Novo Industries, Bagsvaerd, Denmark and the G2 form of the enzyme was purified to homogeneity as previously described (31). Sigma Chemical Co., St. Louis, MO, supplied cyanogen bromide while both iodoacetic acid and N-bromosuccinimide were purchased from Fluka, Buchs, Switzerland. N-Acetyl-L-tryptophan amide was obtained from Bachem, Feinkemikalien AG, Bubendorf, Switzerland. AH-Sepharose 4B was a product of Pharmacia Fine Chemicals, Uppsala, Sweden. Bio-Gel P-100 was from Bio-Rad, Richmond, CA. Substrates and inhibitors were obtained as earlier reported (9) as were reagents and solvents for preparation, isolation and sequencing of peptide fragments (29, 30).

3. METHODS

3.1. Chemical modification with N-bromosuccinimide

The modification of G2 in the presence of specific ligands was performed essentially as previously described (9, 27). Aliquots (200-400 µl) of 10 mM-NBS were sequentially added to a Millipore-filtered solution (50 ml) of G2 (15 µM) containing either acarbose (0.15 mM) or maltose (56 mM) in 50 mM-sodium acetate pH 4.3.

3.2. Affinity chromatography on acarbose-Sepharose

Acarbose was activated with CNBr at pH 10.5 and coupled to AH-Sepharose in 0.2 M-sodium borate pH 8.7 overnight at 4 °C. For affinity chromatography, G2 derivatives were applied to a column of acarbose-Sepharose in 0.1 M-sodium acetate, 0.5 M-sodium chloride pH 4.3. Elution of bound enzyme was achieved with 1.7 M-Tris-HCl pH 7.6. The eluate was immediately dialyzed against 0.05 M-sodium acetate pH 4.3.

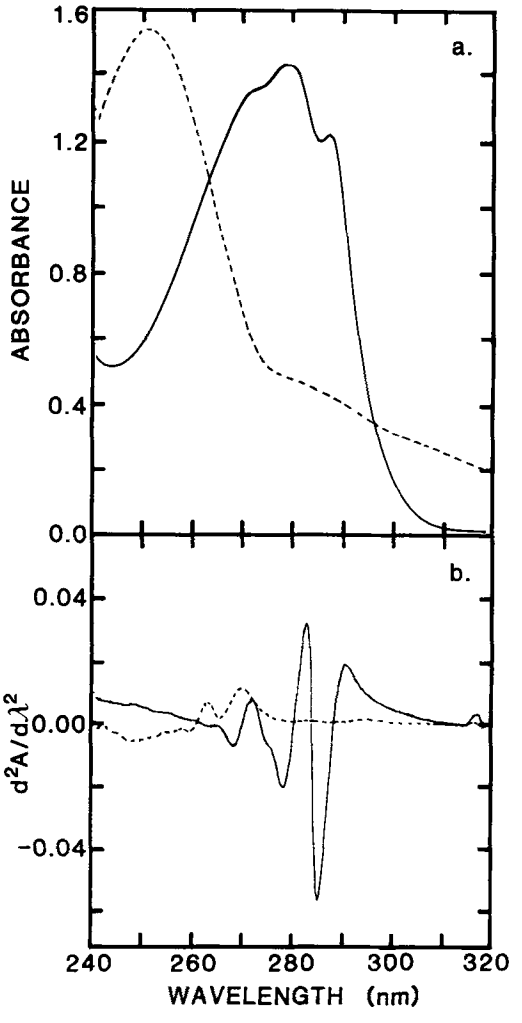

Figure 1. Normal (a) and second-derivative (b) UV-absorption spectra of N-acetyl-tryptophan amide and N-acetyl-oxindolealanine amide. ——, N-acetyl-tryptophan amide (0.26 mM) in 50 mM-sodium acetate pH 4.3. ------, N-acetyl-oxindolealanine amide (0.25 mM) in 50 mM-sodium acetate pH 4.3. The latter spectra were recorded following five minutes of incubation with NBS.

3.3. Preparation and isolation of tryptic fragments

The G2 derivatives were reduced and carboxymethylated essentially as described (31) at a protein concentration of 30 mg×ml^{-1} and a two-fold molar excess of iodoacetate over sulfhydryls. Carboxymethyl-G2 derivatives (5.8 mg×ml^{-1}) were digested at 37 °C with trypsin (0.1

mg×ml^{-1}) for 4 hours (29). Tryptic peptides (40 mg) were separated by gel filtration on Bio-Gel P-100 (1.5×90 cm) in 0.2 M-ammonium bicarbicarbonate pH 7.8 and pooled as earlier published (29). These peptide pools were further fractionated by HPLC using a Waters liquid chromatography system and a Wide-Pore C_{18} column from J.T. Baker Research Products eluted with a linear gradient from 0.1% TFA to 0.1% TFA in 40% 1-propanol (19, 29).

3.4. UV-Spectroscopy

The tryptophan content of peptides was determined spectrophotometrically by the method of SERVILLO et al. (26) employing a Varian 2200 recording spectrophotometer. Figure 1 shows the normal and the 2nd derivative spectra of both N-acetyl-tryptophan amide and N-acetyl-oxindolealanine amide (generated by NBS in situ). It is seen that abolition of the 2nd derivative signal in the region from 275 nm to 320 nm upon NBS oxidation permits the estimation of the content of tryptophan in the presence of oxindolealanine.

3.5. Analytical procedures

Concentrations of G2 were determined spectrophotometrically employing the ε_{280} value of 1.09×10^5 M^{-1}×cm^{-1} (9). Enzymatic activity was determined with maltose (15 mM) as substrate (31). Amino acid analysis of peptides was performed after acid hydrolysis for 24 hours at 110 °C with a Durrum D-500 amino acid analyzer and peptides were sequenced in the presence of Polybrene (17) using a Beckman Sequencer 890C as earlier described (14, 28). Fluorescence spectra were obtained at 25 °C employing an Aminco SPF500 spectrofluorometer.

4. RESULTS

The active and the inactive, oxidized G2 preparations, obtained with acarbose and maltose protection, respectively (section 3.1), contained approx. 10% of non-ligand binding enzyme molecules which were eliminated by affinity chromatography on acarbose-Sepharose (section 3.2). The tryptic fragments of G2 derivatives were obtained and fractionated as described (section 3.3). Peptide fragments, eluting from the Bio-Gel P-100 at a volume between 125 ml and 140 ml, were separated in analytical scale by reverse phase HPLC with monitoring at 280, 250, and 220 nm and gave rise to the chromatograms shown in Figure 2a and b. A large peak indicated by arrows represented a major difference in the UV-absorption properties between fragments derived from the active and inactive G2 derivative, respectively. Both the unmodified and the modified peptide eluted at the same position from the HPLC column. Material corresponding to this peak was isolated on a larger scale from the two G2 derivatives and rechromatographed over the Wide-Pore column using a 60 minute linear gradient from 8 to 16% 1-propanol in 0.1% TFA.

The identification of the isolated fragments was established by amino acid analysis and NH_2-terminal sequencing (Table I). These results are consistent with the fragment:

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Phe-Asn-Val-Asp-Glu-Thr-Ala-Tyr-Thr-Gly-Ser-Trp
109                                         120
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Table I. Amino acid composition of the Phe(109)-Trp(120)-peptide from oxidized glucoamylase G2

G2 derivative	Active	Inactive
Amino acid	Residues/fragment[a]	
Aspartic acid	1.6	1.8
Threonine	1.5	1.7
Serine	1.1	1.3
Glutamic acid	0.9	1.1
Glycine	0.9	1.3
Alanine	1.0	1.0
Valine	0.8	0.7
Tyrosine	0.8	0.8
Phenylalanine	0.7	0.8
Tryptophan[b]	0.9	0.0
Oxindolealanine[c]	-	1.1
NH_2-Terminal yield (%)[d]	70	45

[a] Based on Ala = 1.0.

[b] Estimated from second-derivative UV-absorbance assuming Tyr = 1.0

[c] Estimated from UV-absorbance assuming $\varepsilon_{250} = 6.6\times10^3$ M^{-1}×cm^{-1}.

[d] The yield of phenylthiohydantoin-Phe in the first step of the automated sequencing based on a peptide content determined from amino acid analysis.

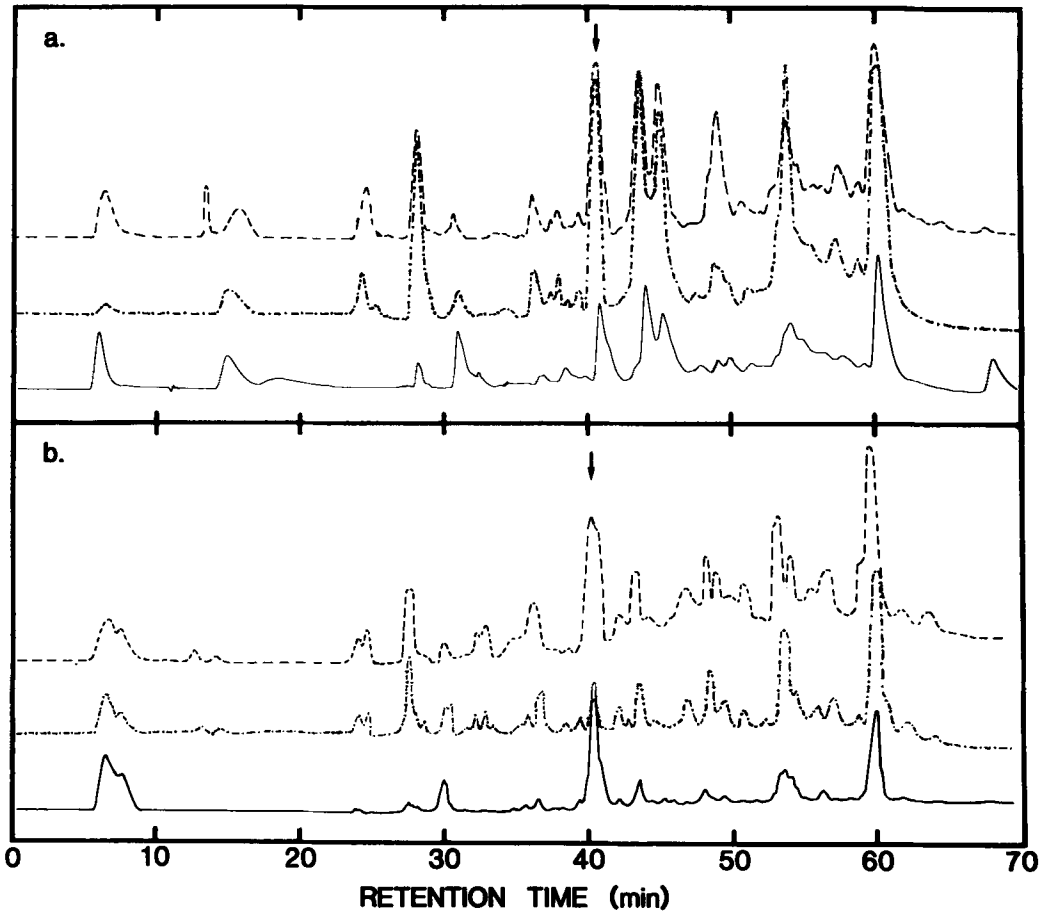

Figure 2. HPLC elution profiles of tryptic fragments from active G2 with four (a) and inactive G2 with five (b) oxidized tryptophans. Approx. 0.4 mg of a mixture of peptides, eluted from a Bio-Gel P-100 column as described in section 4, was applied in 0.1% TFA to a Wide-Pore C_{18} column in each of three separate runs. Elution was accomplished employing an 80 minute linear gradient of 0-40% 1-propanol in 0.1% TFA at a flow rate of 0.5 ml×min^{-1}. The eluant was monitored at 220 nm (------), 250 nm (——) or 280 nm (. – . –). The scale for A_{220} is 10 times greater than that of either A_{250} or A_{280}. The arrows indicate the peak representing a major change in the A_{280}/A_{250} ratio between the profiles in a and b.

in the amino acid sequence of glucoamylase (5, 30). The UV-absorption of the isolated peptide (Figure 3) confirmed the presence of an intact tryptophan in the fragment from G2 oxidized in the presence of acarbose, while in contrast the fragment from the inactive G2 derivative exhibited oxindolealanine UV-absorption. Minor components from digests of both inactive and active G2 derivatives comprising residues Phe(109)-Arg(122) and Phe(109)-Arg(125) (30) were also isolated and possessed similar UV-spectra to those above (results not shown). Thus, the oxidation of the Trp(120) seemed to be accompanied by the loss of maltose-hydrolyzing capacity of glucoamylase.

The intrinsic fluorescence of the G2 complexes with the inhibitor acarbose (a pseudotetrasaccharide), with starch or with the small substrates maltose and maltotriose are shown in Figure 4. Acarbose induced a red shift and an increase of the relative fluorescence of G2, whereas all the substrates, i.e. starch, maltose and maltotriose, partially quenched the fluorescence and induced a blue shift of the maximum.

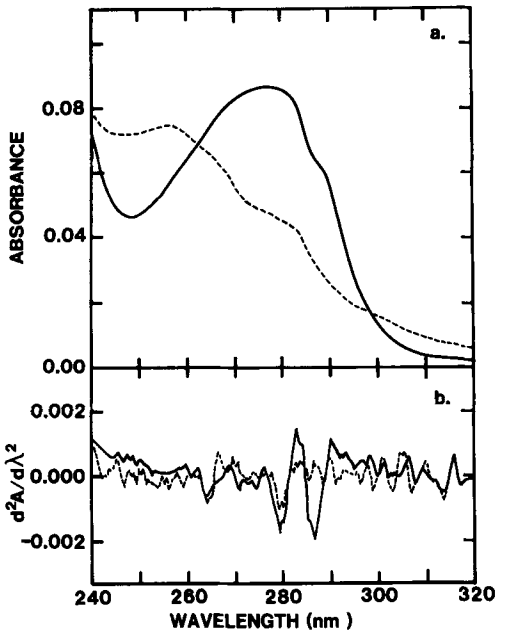

Figure 3. Normal (a) and second-derivative (b) UV-absorption spectra of the isolated Phe(109)-Trp(120)-peptide from active, oxidized G2 (16.7 μM,——) and inactive, oxidized G2 (14,5 μM,------) in 100 mM-ammonium bicarbonate pH 7.8.

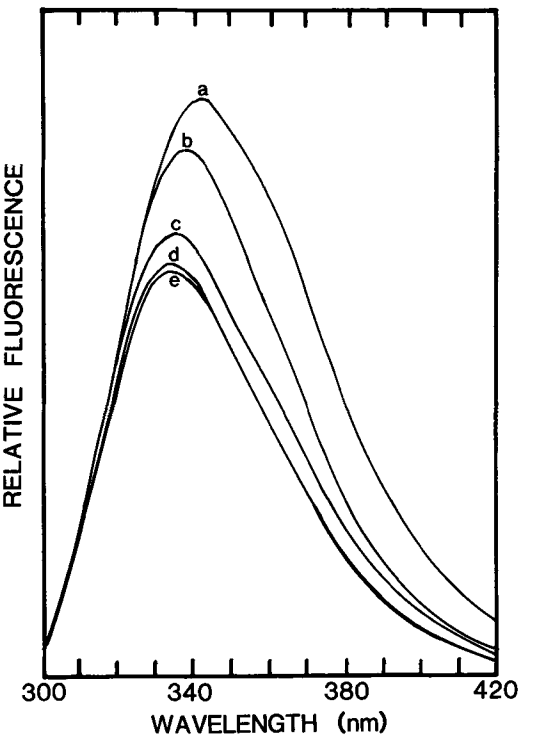

Figure 4. Fluorescence emission spectra of native glucoamylase G2 in the absence (b) and presence of ligands. Enzyme (3.7 μM) in 50 mM-sodium acetate pH 5.0 at 25 °C in the presence of 0.14 mM-acarbose (a), 0.25% soluble starch (c), 15 mM-maltose (d) or 15 mM-maltotriose (e). Excitation wavelength was fixed at 280 nm.

5. DISCUSSION

It has previously been shown that N-bromosuccinimide oxidation of glucoamylases G1 and G2 from A. niger led to derivatives that were devoid of enzymatic activity. The presence of the inhibitor acarbose afforded protection of both the enzymatic activity and two tryptophanyl residues. All other ligands examined only protected a single tryptophanyl residue, thought to be located in subsite 1 of the substrate binding region (9, 11), and in these cases the resulting derivatives were catalytically inactive (9). Thus, the tryptophan essential for hydrolase activity is oxidized in the latter derivatives and three tryptic peptides (29, 30) containing the oxidized tryptophan: Phe(109)-Trp(120), Phe(109)-Arg (122), and Phe(109)-Arg(125) were isolated. The first one was predominant presumably as a consequence of both a slow hydrolysis of the Arg(122)-Pro(123) peptide bond and a contamination of the employed commercial trypsin preparation with chymotrypsin. The UV-absorption properties of the different isolated peptides, however, clearly confirmed the complete oxidation of Trp(120) by NBS in the presence of maltose and the protection of this residue in the G2-acarbose complex. Since no major difference was observed for other tryptophanyl residues when comparing the Bio-Gel P-100 and the HPLC chromatograms of tryptic fragments from the active and inactive G2 derivatives, the Trp(120) was concluded to be essential for maintaining the catalytic activity. However, its precise role in the function of glucoamylase remains unclear. Thus, this residue was not protected from oxidation by small substrates like maltose and maltotriose, but seemed on the contrary to be even more readily oxidized in

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Glucoamylase from Aspergillus niger (5, 30):
                                                    120
-Pro-Lys-Phe-Asn-Val-Asp-Glu-Thr-Ala-Tyr-Thr-Gly-Ser-Trp-Gly-Arg-Pro-


Taka-amylase A from Aspergillus oryzae (33):
                                                    83
-Pro-Gln-Asp-Cys-Ala-Tyr-Gly-Asp-Ala-Tyr-Thr-Gly-Tyr-Trp-Glu-Thr-Asp-
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Figure 5. Sequence alignment guided by the essential Trp(120) of glucoamylase and the Trp(83) of Taka-amylase A, involved in substrate binding (20).

their presence, as judged from the loss of activity (9). Furthermore, SAVEL'EV and FIRSOV have recently reported that the rate of NBS inactivation of glucoamylase from Aspergillus awamori is significantly reduced by maltotetraose, but not by smaller substrates, and this suggests a tryptophan to be located in subsite 4 of this glucoamylase (25). Similarly, in our studies of A. niger glucoamylase starch afforded some protection against oxidation, although only acarbose completely prevented the inactivation under the actual experimental conditions (9). Acarbose and starch thus seem to interact in different ways with G2 which was consistent with the clearly different changes of the G2 intrinsic fluorescence spectra induced by each of these compounds. In summary, the Trp(120) appears to be exposed in enzyme-substrate complexes comprising the binding affinity subsites 1, 2, and 3 (9, 11), partially protected in complexes with longer substrates and effectively protected only in the inactive enzyme-acarbose complex (9). Since the hydrolysis occurs between sugar residues number 1 and 2 from the non-reducing end of the substrate (11), the experiments with glucoamylase from A. niger also indicate that the essential Trp(120) is probably located at some distance from the catalytic site. It might, however, be involved in maintaining the structural integrity needed for productive substrate binding and catalysis to occur. Thus, the catalytically inactive G2, prepared by oxidation in the presence of the small substrate maltose, still does bind substrate (9) and an analogous situation has been described in structural detail for lysozyme after oxidation of Trp(62) to oxindolealanine. In this case a conformational change caused oxindolealanine(62) to block a part of the active site cleft and as a consequence ligands would bind but non-productively (4).

Although there is no general sequence homology between this glucoamylase and known α-amylases (10, 18, 24, 32, 33), it was observed that a short stretch of polypeptide chain, preceding Trp(120) in glucoamylase and the Trp(83) in the Taka-amylase A (20) (from A. oryzae) was identical (Figure 5). The Trp(83) is located in the active site cleft of the three-dimensional structure of Taka-amylase A and model fitting studies with amylose have recently proposed that Trp(83) participates in binding of this substrate (20). Although this tryptophan is not conserved throughout the α-amylases, it is present in the two fungal enzymes, Taka-amylase A and glucoamylase, and one might speculate about similarities in their structures. In fact, preliminary secondary structure predictions a.m. CHOU and FASMAN (8), indicate that glucoamylase might fold in two domains with a COOH-terminal region dominated by β-structure and a large NH_2-terminal domain of alternating α- and β-structure (B. SVENSSON, unpublished data). Thus the exo-α-glucanase, glucoamylase, perhaps belongs to the class of enzymes having an α/β-barrel supersecondary structure (21), like the endo-α-glucanases Taka-amylase A (20) and porcine pancreas α-amylase (7). It is of great interest to test this hypothesis by using the coordinates obtained from X-ray crystallography of the α-amylases to guide the folding of the polypeptide chain of glucoamylase.

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REFERENCES

1. BACHMEYER, H.: Effect of tryptophan modification on the activity of bacterial and viral neuraminidase. FEBS Lett. 23, 217-219 (1972)
2. BARKER, S.A., C.J. GRAY & M.E. JOLLEY: Photooxidation of glucoamylase I from Aspergillus niger. Biochem. Biophys. Res. Comm. 45, 654-661 (1971)
3. BEDDELL, C.R., C.C.F. BLAKE & S.J. OATLEY: An X-ray study of the structure and binding properties of iodine-inactivated lysozyme. J. Mol. Biol. 97, 643-654 (1975)
4. BLAKE, C.C.F., R. CASSELS, C.M. DOBSON, F.M. POULSEN, R.J.P. WILLIAMS & K.S. WILSON: Structure and binding properties of hen lysozyme modified at tryptophan 62. J. Mol. Biol. 147, 73-95 (1981)
5. BOEL, E., I. HJORT, B. SVENSSON, F. NORRIS, K.E. NORRIS & N.P. FIIL: Glucoamylases G1 and G2 from Aspergillus niger are synthesized from two different but closely related mRNAs. EMBO J. 3, 1097-1102 (1984)
6. BOEL, E., M. TRIER HANSEN, I. HJORT, I. HØEGH & N.P. FIIL: Two different types of intervening sequences in the glucoamylase gene from Aspergillus niger. EMBO J. 3, 1581-1585 (1984)
7. BUISSON, G., E. DUÉE, R. HASER & F. PAYAN: Crystallographic structure of pig pancreas α-amylase (2.9 Å resolution). 8th International Biophysic Congress, Bristol (1984), abstract 112, p. 60.
8. CHOU, P.Y. & G.D. FASMAN: Prediction of protein conformation. Biochemistry 13, 222-244 (1974)
9. CLARKE, A.J. & B. SVENSSON: The role of tryptophanyl residues in the function of Aspergillus niger glucoamylase G1 and G2. Carlsberg Res. Commun. 49, 111-122 (1984)
10. HAGENBÜCHLE, O., R. BOVEY & R.A. YOUNG: Tissue-specific expression of mouse α-amylase genes: nucleotide sequence of isoenzyme mRNAs from pancreas and salivary gland. Cell 21, 179-187 (1980)
11. HIROMI, K., M. OHNISHI & A. TANAKA: Subsite structure and ligand binding mechanism of glucoamylase. Mol. Cell. Biochem. 51, 79-95 (1983)
12. IMOTO, T., L.N. JOHNSON, A.C.T. NORTH, D.C. PHILLIPS & J.A. RUPLEY: Vertebrate lysozymes. Enzymes 3rd ed. 7, 665-868 (1972)
13. INOKUCHI, N., T. TAKAHASHI, A. YOSHIMOTO & M. IRIE: N-Bromosuccinimide oxidation of a glucoamylase from Aspergillus saitoi. J. Biochem. 91, 1661-1668 (1982)
14. JOHANSEN, J.T., C. OVERBALLE-PETERSEN, B. MARTIN, V. HASEMANN & I. SVENDSEN: The complete amino acid sequence of copper, zinc superoxide dismutase from Saccharomyces cerevisiae. Carlsberg Res. Commun. 44, 201-217 (1979)
15. JOLLEY, M.E. & C.J. GRAY: Tryptophanyl and carboxylic acid residues in the active center of glucoamylase I from Aspergillus niger. Carbohyd. Res. 49, 361-370 (1976)
16. KITA, Y., M. FUKAZAWA, Y. NITTA & T. WATANABE: Kinetic study on chemical modification of Taka-amylase A. I. Location and role of tryptophan residues. J. Biochem. 92, 653-659 (1982)
17. KLAPPER, D.G., C.E. WILDE III & J.D. CAPRA: Automated amino acid sequence of small peptides utilizing Polybrene. Anal. Biochem. 85, 126-131 (1978)
18. KLUH, I.: Amino acid sequence of hog pancreatic α-amylase isoenzyme I. FEBS Lett. 136, 231-234 (1981)
19. MAHONEY, W.C. & M.A. HERMODSON: Separation of large denatured peptides by reverse phase high performance liquid chromatography. Trifluoroacetic acid as a peptide solvent. J. Biol. Chem. 255, 11199-11203 (1980)
20. MATSUURA, Y., M. KUSUNOKI, W. HARADA & M. KAKUDO: Structure and possible catalytic residues of Taka-amylase A. J. Biochem. 95, 697-702 (1984)
21. MUIRHEAD, H.: Triose phosphate isomerase, pyruvate kinase and other α/β-barrel enzymes. Trends Bioch. Sci. 8, 326-330 (1983)
22. NITTA, Y., T. KUNIKATA & T. WATANABE: Difference spectroscopic study of the interaction between soybean β-amylase and substrate or substrate analogues. J. Biochem. 93, 1195-1201 (1983)

23. OHNISHI, M., M. TANIGUCHI & K. HIROMI: Kinetic discrimination of tryptophan residues of glucoamylase from Rhizopus niveus by fast chemical modification with N-bromosuccinimide. Biochim. Biophys. Acta 744, 64-70 (1983)
24. ROGERS, J.C. & C. MILLIMAN: Isolation and sequence analysis of a barley α-amylase cDNA clone. J. Biol. Chem. 258, 8169-8174 (1983)
25. SAVEL'EV, A.N. & L.M. FIRSOV: Effect of modification of some amino acid radicals on enzymatic activity of glucoamylase from Aspergillus awamori. Biokhimiya 48, 1311-1318 (1983)
26. SERVILLO, L., G. COLONNA, C. BALESTRIERI, R. RAGONE & G. IRACE: Simultaneous determination of tyrosine and tryptophan residues in proteins by second-derivative spectroscopy. Anal. Biochem. 126, 251-257 (1982)
27. SPANDE, T.F. & B. WITKOP: Determination of the tryptophan content of proteins with N-bromosuccinimide. Meth. Enzymol. XI, 498-506 (1967)
28. SVENDSEN, I., B. MARTIN & I. JONASSEN: Characteristics of Hiproly barley III. Amino acid sequences of two lysine-rich proteins. Carlsberg Res. Commun. 45, 79-85 (1980)
29. SVENSSON, B., K. LARSEN & I. SVENDSEN: Amino acid sequence of tryptic fragments of glucoamylase G1 from Aspergillus niger. Carlsberg Res. Commun. 48, 517-527 (1983)
30. SVENSSON, B., K. LARSEN, I. SVENDSEN & E. BOEL: The complete amino acid sequence of the glycoprotein, glucoamylase G1 from Aspergillus niger. Carlsberg Res. Commun. 48, 529-544 (1983)
31. SVENSSON, B., T.G. PEDERSEN, I. SVENDSEN, T. SAKAI & M. OTTESEN: Characterization of two forms of glucoamylase from Aspergillus niger. Carlsberg Res. Commun. 47, 55-69 (1982)
32. TÄKKINEN, K., R.F. PETTERSSON, N. KALKINEN, I. PALVA, H. SÖDERLUND & L. KÄÄRIÄNEN: Amino acid sequence of α-amylase from Bacillus amyloliquefaciens deduced from the nucleotide sequence of the cloned gene. J. Biol. Chem. 258, 1007-1013 (1983)
33. TODA, H., K. KONDO & K. NARITA: The complete amino acid sequence of Taka-amylase A. Proc. Japan Acad. 58, Ser. B, 208-212 (1982)
34. TRUSCHEIT, E., W. FROMMER, B. JUNGE, L. MÜLLER, D.D. SCHMIDT & W. WINGENDER: Chemie und Biochemie mikrobieller α-Glucosidaseninhibitoren. Angew. Chem. 93, 738-755 (1981)

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