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# RELATIONSHIP BETWEEN THE DISTRIBUTION OF THE CHAIN LENGTH OF AMYLOPECTIN AND THE CRYSTALLINE STRUCTURE OF STARCH GRANULES

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## ABSTRACT

The distributions of chain lengths in the amylopectins of starches from 20 species (11 A-, 6 B-, and 3 C-type) were characterised by h.p.l.c. in terms of the relationship between the molecular structure of the amylopectin and the crystalline structure of the starch granule. The weight-average chain-lengths of the amylopectins of the A-, B-, and C-type starches were in the ranges 23–29, 30–44, and 26–29, respectively. Gel-permeation chromatograms of the amylopectins debranched with isoamylase showed bimodal distributions of fractions containing long and short chains for 17 specimens (including corn, rice, potato, *etc.*) and trimodal distributions, of which the fraction containing short chains had twin peaks, for wheat, tapioca, and tulip amylopectins. The correlation coefficients between the average chain-lengths of amylopectins and the fractions of long and short chains and the ratio of the fractions of short and long chains by weight were 0.90, 0.69, and −0.95, respectively. In general, amylopectin molecules of A-type starches have shorter chains in both the long- and short-chain fractions and larger amounts of the short-chain fractions than those of the B-type starches. The chain lengths of amylopectins of the C-type starches were intermediate and it is inferred that these starches possibly yield any type of crystalline structure depending on the environmental temperature and other factors, whereas the A- and B-type starches are insensitive to temperature.

## INTRODUCTION

The molecular arrangements of starch granules are classified into three types, A–C, by X-ray powder diffraction[1]. These crystalline varieties appear to be specific for different botanical sources, although alteration depending on the environmental temperature has been observed for those from soybean[2] and sweet potato[3]. Recently, we suggested that the production of crystalline varieties is determined by the chain length of amylopectin, on the basis of the finding that the chain lengths of amylopectins of A-type starches are shorter than those for B-type starches and,

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in addition, amylodextrins with short and long chains have relatively higher tendencies to crystallise into the A and B types, respectively[4].

Amylopectin and glycogen are (1→4),(1→6)-linked α-D-glucans which differ in the distribution of chain length and in the state of molecular association (crystalline or amorphous). Amylopectin contains both long and short (1→4)-linked chains, whereas glycogen contains a single type of chain[5–9]. We now report the characterisation of the distribution of chain lengths in amylopectins from various sources, in connection with the crystalline polymorphs of starch granules. We have used a new technique involving high-performance gel-permeation chromatography monitored with a low-angle laser-light-scattering photometer and a differential refractometer. This technique has been used in the analyses of the distribution of chain lengths in amyloses[10,11]

## EXPERIMENTAL

*Materials.* — Starch specimens were the same as those used previously[4], except for amylomaize starch which was a generous gift from Dr. T. Yamada (Mie University). Crystalline isoamylase and pure cyclosophoroheptadecaose[12] were kindly donated by Professor T. Harada (Kobe Women's University) and Professor K. Koizumi (Mukogawa Women's University), respectively. Fractionated specimens of pullulan for standards were products of the Hayashibara Biochemical Institute (donated by Dr. Y. Tsujisaka).

*Debranching amylopectin.* — The linear chains of amylopectin were prepared by debranching starch with isoamylase, without pre-fractionation of the amylopectin. Suspensions of other than amylomaize starch (55 mg wet basis, 12–20% moisture content) in water (3.75 mL) were kept at 100° for 6 min. A solution of amylomaize starch in dimethyl sulfoxide (0.5 mL) was diluted to 3.75 mL with water with heating at 100°. The resulting pastes were mixed with M acetate buffer (pH 3.5, 0.25 mL) and 2.1 U (1–5 μL) of isoamylase, and then incubated at 45°. The hydrolysis was complete after incubation for 1 h, but it was continued for 2.5 h. Isoamylase was freed[13] from alpha-amylase and α-D-glucosidase. After the addition of 0.5M sodium phosphate buffer (1 mL) containing 0.1% of sodium azide, the mixture was heated for 3 min at 100° to inactivate the enzyme, then mixed with 1-butanol (0.45 mL), and incubated at 30° for 2 h to precipitate the 1-butanol complex of the long chains from amylose. The precipitate was removed by centrifugation at 600*g* for 5 min, and the supernatant solution was subjected to gel chromatography as described below. The average chain-length of amylopectin determined by this treatment, without pre-separation, agreed well with that determined on the pre-separated amylopectin from starch[4]. Furthermore, prior to this experiment, we confirmed also that specimens of potato, sweet-potato, and kuzu starches prepared by this isoamylase–butanol treatment gave distributions of chain lengths which were almost identical with those of debranched, pre-separated amylopectins by the following permeation chromatography.

*H.p.l.c.* — Similar instrumentation to that described in previous studies[10,11] was used. A stainless-steel tube (0.1 mm × 2 m) was inserted between an accumulator and the injector valve of a Toyo Soda 803C chromatograph to smooth the flow. The sample solution (1 mL) was filtered through a 0.22-$\mu$m filter unit (MILLEX-GS, Millipore), injected into the chromatograph, and pumped into a guard column (TSK-PWH) followed by three main columns. TSK-GEL G3000PW (× 2) and G2000PW, connected in that sequence. The main columns were kept at 37°. The column eluate was monitored first with a flow-through cell (30 $\mu$L) in a Toyo Soda LS-8 laser-light-scattering photometer (He–Ne laser, 5-mW output, 5° scattering angle) and then with one (10 $\mu$L) in a Toyo Soda RI-8011 differential refractometer. The responses of these detectors were recorded with a two-pen recorder (chart speed, 4 mm/min). The operation ranges of the photometer and refractometer were set at 32. The eluent was 100mM sodium phosphate buffer (pH 6.2) containing 0.02% of sodium azide; the flow rate was 0.52 mL/min, but it varied occasionally between 0.505–0.525.

*Data analysis.* — If light-scattering measurement of a polymer solution is performed with a dilute solution of which the second virial coefficient is negligibly small, and at a low scattering angle near to zero where the error due to the angular dependence may be ignored, the weight-average molecular weight of the polymer is given[10,11] by Eq. *1*.

$$M_w = k' \frac{(LS)}{(RI)} \Big/ \frac{(dn)}{(dc)} = k \frac{(LS)}{(RI)}, \qquad 1$$

where (LS) and (RI) denote the detector responses of the photometer and refractometer, respectively, (dn/dc) is the refractive index increment of the polymer, and $k'$ and $k$ denote an instrumental constant and $k'$/(dn/dc), respectively. The value of $k$ can be obtained from the detector responses for a standard polymer of known molecular weight and the same refractive increment as for the specimen. Fractionated specimens of pullulan with specified narrow distributions of molecular weight are suitable as such standards[10,11,14]. Fractions P-10 and P-5 were used, for which the molecular weights determined by this technique, using cyclosophoroheptadecaose as the standard, were 10,200 and 5,300, respectively. These values agreed well with those determined previously using bovine serum albumin and hen ovalbumin as standards.

The detector responses for the whole or particular parts of specimens were measured by excision and weighing of the peaks on photocopies of the recorder chart obtained with both monitors, and those at specified retention times were measured by length.

The apparent time lag observed between the traces of the photometer and the refractometer due to the positional differences of the two monitors in the flow-line and the pens in the recorder was corrected for by the peak positions of cyclosophoroheptadecaose with the two monitors.

RESULTS AND DISCUSSION

Theoretically, Eq. *1* is confined to the scattering intensity measured with an infinitely dilute solution at zero-scattering angle ($\theta$). The difficulties of such measurements are overcome by conventional extrapolations of the data at several angles and concentrations with Zimm plots[15]. Previous studies of amylose[11] showed that the error due to the measurement at $\theta = 5°$ with a photometer could be ignored, and this is safe here because the chains of debranched amylopectin are considerably smaller than those of normal amylose molecules. However, due to the small molecules, the concentration of the sample solution had to be increased to $\geqslant$0.4% to obtain the proper scattering intensity for accurate analysis. The error due to the concentration was examined with, for example, waxy-rice starch in the concentration range 0.4–1.1%, and was too small to detect because the (LS)/(DR) value was constant within experimental error.

Fig. 1 shows a gel-permeation chromatogram of the debranched chains of waxy-rice amylopectin with monitoring by a low-angle laser-light-scattering photometer and a differential refractometer. The results were reproducible. The chromatogram with the refractometer shows the bimodal distribution of the chain length, and a similar distribution has been reported previously[5,8,16–22]. The small peak at the tail of the elution with the refractometer was due to ammonium sulphate incorporated with the isoamylase (indicated by the arrow in Fig. 1). The

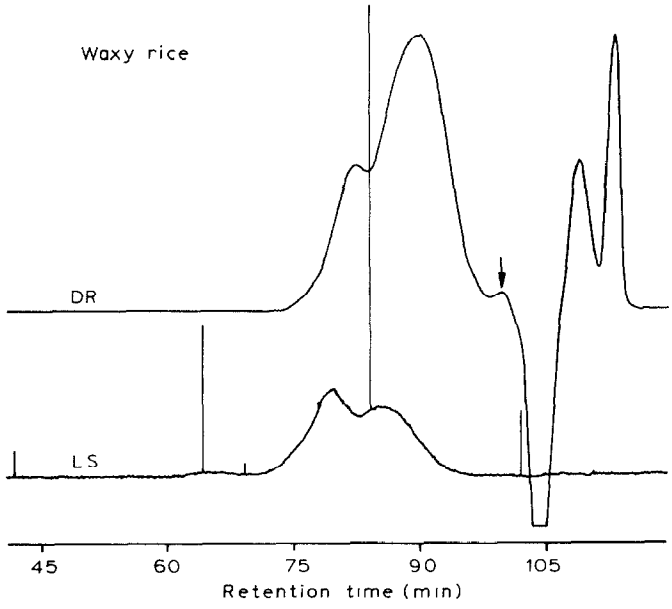
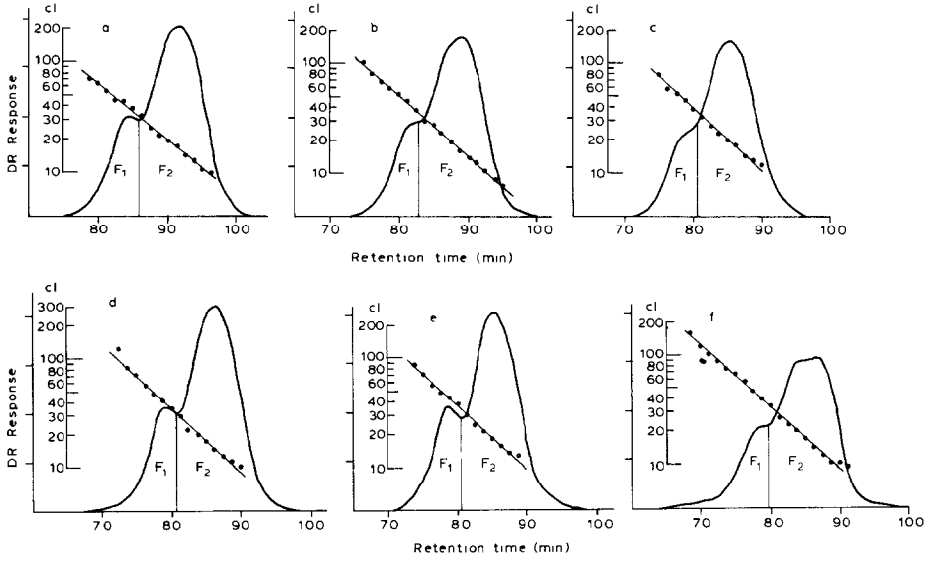



Fig. 1 Gel-permeation chromatogram of debranched waxy-rice starch monitored with a low-angle laser-light-scattering photometer (LS) and a differential refractometer (DR). The arrow indicates the elution of ammonium sulphate (see text).

photometer trace occasionally showed a peak of variable intensity at the void volume (~65 min), but there was no corresponding peak with the refractometer, suggesting the presence of a very small amount of so-called micro-gels, amylose or undebranched amylopectin, but this was not examined further. The debranched chains were eluted in the range 70–100 min retention time, but ~3 h were required for each measurement because of the slow elution of acetate and 1-butanol from the cross-linked hydrophilic vinyl polymer[23]. Since the responses of the refractometer and the photometer are proportional to the concentration and the product of the concentration and the molecular weight, respectively, the molecular weight of the material in the eluate at a certain retention time can be calculated, using Eq. *1*, from the responses with the two monitors after correction for the time lag between the monitors.

Fig. 2. shows the chain-length distribution-profiles of amylopectins from various sources. They are shown on a weight-basis rather than the numerical-basis as suggested by Palmer *et al.*[9], because the former allows calculation of the average chain-lengths of the whole or any particular subfraction. In addition, it is convenient to compare the results with those previously reported[5–9]. Most of the specimens showed a bimodal distribution-profile similar to that of waxy-rice starch, whereas wheat, tapioca, and tulip amylopectins showed trimodal distributions. Similar distributions have been reported[24] for wheat and barley starches. The form of the chain-length distribution-profile appears to be characteristic of a species, because rice and wheat starches from six and seven cultivars, respectively, showed bimodal and trimodal distributions[25]. Since both the slow-elution peaks of the trimodal profiles appear to correspond to the F2 group of the bimodal specimens, the eluate was divided into two subfractions, F1 and F2, at the minimum of the elution curve with the refractometer irrespective of the distribution modes as shown



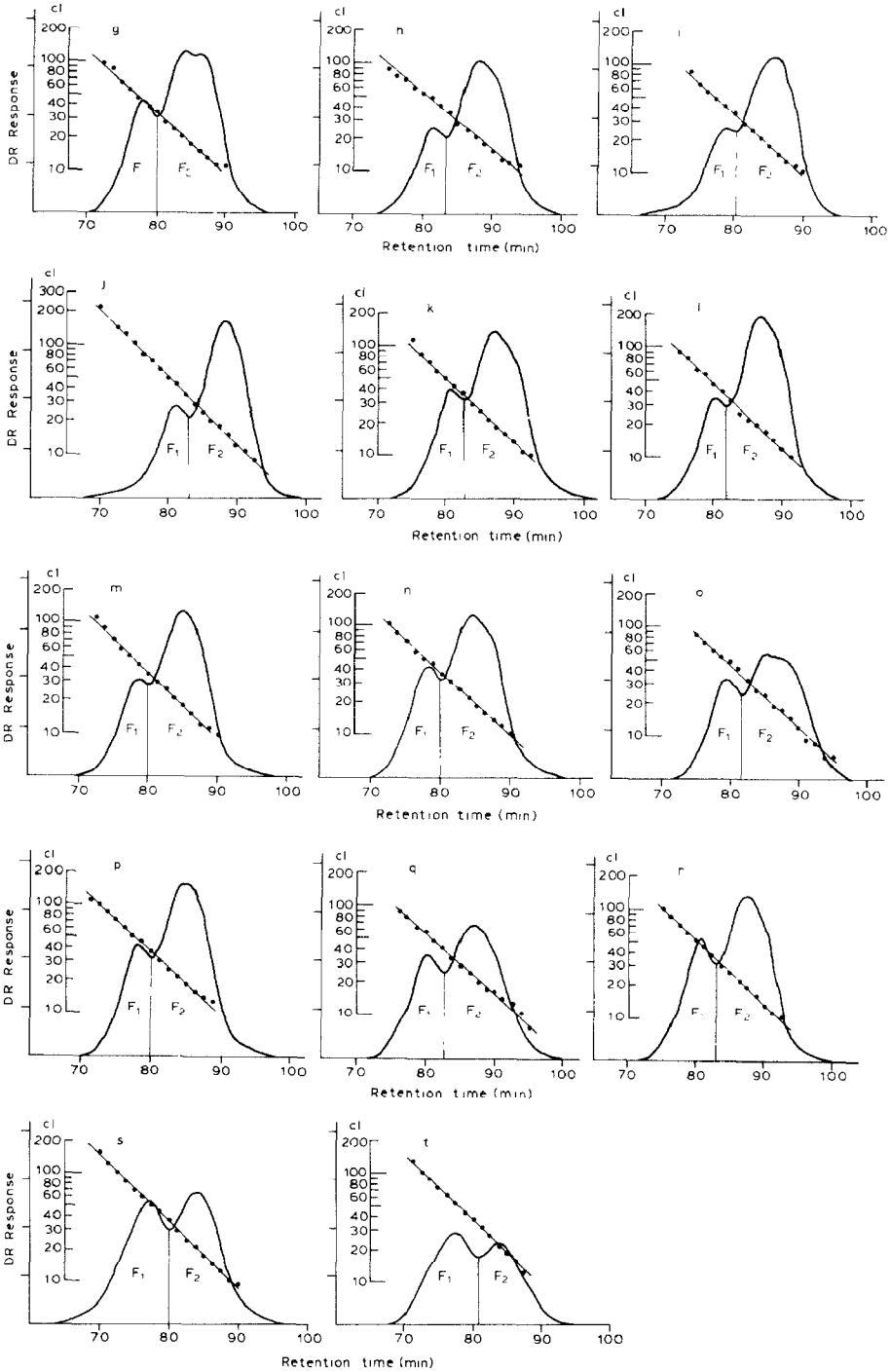



Fig 2. Distributions of chain lengths of amylopectins from various starches; for identification of a–t, see Table I.

in Fig. 2. The weight-average c.l. of F1 and F2, the c.l. at the peak maxima and the boundary between F1 and F2, as well as the weight-average c.l. of whole amylopectin, are listed in increasing order in Tables I and II. These measurements were repeated independently at least three times per specimen and the average values are shown.

The chain-length-distribution patterns (Fig. 2) were specific for the sources and seemed to be independent of each other, but the following regular changes were found. The correlation coefficients between the average chain-length of whole

TABLE I

CHARACTERISATION OF THE DISTRIBUTION OF THE CHAIN LENGTH OF AMYLOPECTIN

| *Source* | *Average chain-length (c.l.)* | | | *Chain length (c.l.)* | | | $M_w^c/M_n^d$ | *Section in Fig. 2* |
|---|---|---|---|---|---|---|---|---|
| | *Whole* | *F1* | *F2* | *F1 (Max.)*[a] | *Boundary*[b] | *F2 (max.)*[a] | | |
| *A-Type* | | | | | | | | |
| Waxy rice | 23 | 48 | 17 | 41 | 33 | 16 | 1.4 | a |
| Waxy corn | 24 | 51 | 18 | 39 | 36 | 15 | 1.3 | b |
| Iris | 25 | 50 | 18 | 37 | 33 | 18 | 1.3 | c |
| Rice, Japonica | 25 | 56 | 16 | 40 | 33 | 15 | 1.4 | d |
| Taro (Satoimo) | 25 | 51 | 17 | 43 | 34 | 16 | 1.3 | e |
| Wheat | 25 | 56 | 17 | 38 | 34 | 19<br>13 | 1.4 | f |
| Tapioca | 26 | 52 | 17 | 45 | 34 | 19<br>14 | 1.4 | g |
| Gladiolus | 27 | 53 | 18 | 46 | 35 | 19 | 1.3 | h |
| Corn | 28 | 60 | 17 | 41 | 33 | 15 | 1.4 | i |
| Rice, Indica | 28 | 59 | 17 | 43 | 32 | 16 | 1.5 | j |
| Sweet potato (Minamiyutaka) | 29 | 57 | 18 | 46 | 35 | 19 | 1.4 | k |
| Average | 26 | 54 | 17 | 42 | 34 | 16 | | |
| *C-Type* | | | | | | | | |
| Kuzu | 26 | 53 | 17 | 44 | 35 | 18 | 1.3 | l |
| Yam (Nagaimo) | 28 | 55 | 19 | 44 | 37 | 18 | 1.3 | m |
| Sweet potato (Norin 2) | 30 | 55 | 19 | 47 | 37 | 20 | 1.5 | n |
| Average | 28 | 55 | 18 | 45 | 36 | 19 | | |
| *B-Type* | | | | | | | | |
| Tulip | 30 | 56 | 18 | 48 | 35 | 21<br>16 | 1.4 | o |
| Lotus | 30 | 56 | 20 | 47 | 36 | 19 | 1.4 | p |
| Lily | 34 | 61 | 20 | 50 | 37 | 20 | 1.5 | q |
| Potato | 34 | 62 | 20 | 50 | 36 | 20 | 1.5 | r |
| Canna | 44 | 73 | 19 | 49 | 34 | 19 | 1.6 | s |
| Amylomaize | 44 | 65 | 19 | 50 | 33 | 21 | 1.4 | t |
| Average | 36 | 62 | 19 | 49 | 35 | 19 | | |

[a]Maximum of the elution peak with DR (see Fig. 2). [b]Between F1 and F2. [c]Weight-average molecular weight. [d]Number-average molecular weight.

TABLE II

RATIOS OF SUBFRACTIONS (F2/F1)

| *Source* | *Ratio of subfractions (F2/F1)* | |
|---|---|---|
| | *(Weight basis)* | *(Molar basis[a])* |
| *A-Type* | | |
| Waxy rice | 3.6 | 11 |
| Waxy corn | 3.9 | 11 |
| Iris | 3.6 | 10 |
| Rice, Japonica | 3.5 | 12 |
| Taro (Satoimo) | 3 2 | 10 |
| Wheat | 3.6 | 12 |
| Tapioca | 2.7 | 8 |
| Gladiolus | 3.0 | 9 |
| Corn | 2.9 | 10 |
| Rice, Indica | 2.6 | 9 |
| Sweet potato (Minamiyutaka) | 2.6 | 8 |
| Average | 3.2 | 10 |
| *C-Type* | | |
| Kuzu | 3.1 | 9 |
| Yam Nagaimo) | 2.9 | 9 |
| Sweet potato (Norin 2) | 2.5 | 7 |
| Average | 2 8 | 8 |
| *B-Type* | | |
| Tulip | 2.3 | 7 |
| Lotus | 2.6 | 7 |
| Lily | 2.0 | 6 |
| Potato | 1 9 | 6 |
| Canna | 1 1 | 4 |
| Amylomaize | 0.8 | 3 |
| Average | 1.8 | 6 |

[a]Calculated by $[F1(\overline{c.l.})]/[F2(\overline{c.l.})] \times F2/F1$ (by weight). Average chain-lengths (c.l.) of F1 and F2 should be number-average, but here weight-average values are used. The error due to this is not significant.

amylopectin and those of subfractions F1 and F2 were 0.90 and 0.69, respectively, as shown in Figs. 3 and 4. It is concluded that the average chain-length of amylopectin is dependent mainly on the chain length of the larger chain component, F1, which probably comprises mostly B-chains (B-chains are linked to two or more other chains, whereas an A-chain is bound to a single other chain by its reducing group[26]). However, if the two points of greatest chain-length of amylopectin in Fig. 4 are eliminated, the correlation coefficient increases to 0.84. Therefore, more data on diverse kinds of starch are necessary for a final conclusion. Fig. 5 shows the correlation between the average chain-length of amylopectin and the relative amounts of subfractions F1 and F2 (F2/F1). The correlation coefficient was −0.95, indicating that amylopectins with larger average chain-lengths have smaller proportions of the shorter chain fraction, F2. These correlations imply that there is a

regular spectrum-like change in the distributions of chain lengths of amylopectins of different species, namely, amylopectins with larger average chain-lengths have longer chains in both F1 and F2 and smaller proportions of F2. These regular changes suggest that a fine control mechanism, which differs slightly with the species, is involved in the biosynthesis of amylopectin. This regular variation of the molecular structure of amylopectin is of interest for understanding the functional properties of starches. The upper limit of the chain length of F1 chains was ~100 in most starches and ~200 or more in some starches (wheat, Indica rice, corn, amylomaize, and canna), and the chain lengths of the peak maxima were in the range 37–50. The chain lengths at the boundary of F1 and F2 were in the range 32–37 and there was no correlation between these values and the average chain-length of whole amylopectin (the correlation coefficient was 0.02). The peak maxima of the F2 chains were distributed in the range 15–21. The lower limit for the F2 chains was not determined, but it appears[21,24,26,28] to be 6. It is concluded that, generally, the chain length of the F1 chains ranges from 35 to 100 (and occasionally to 200 or more), and that the F2 chains vary from 6 to 35.

The weight-average chain-lengths of the eleven A-type starches ranged up to 29 and the average value was 26, whereas those of the six B-type starches went beyond the upper limit of the A-type and the average value was 36. This confirms our previous finding that A-type starches have shorter chains than B-type starches. The values for the C-type starches, though only three kinds were examined, were in the range 26–30, which were near the upper limit values of the A-type starches. In this range, the A- and B-type starches are also included. The author considers

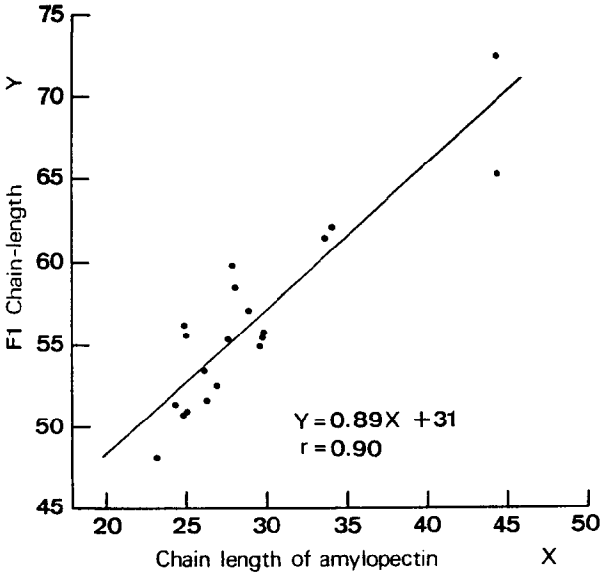



Fig. 3. Relationship between chain lengths of amylopectin and its subfraction F1. Average chain-lengths (to one decimal place) are plotted to avoid overlap of spots.

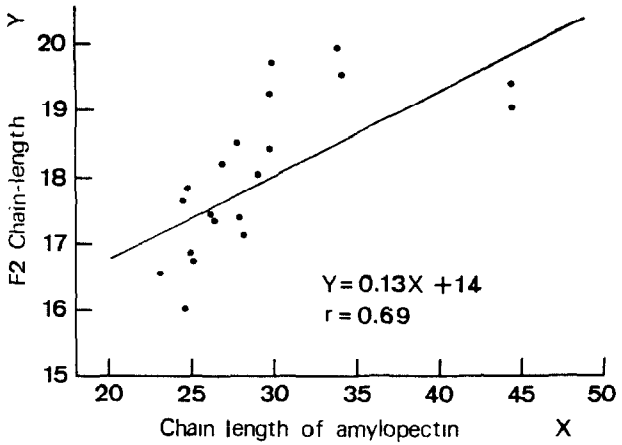



Fig. 4. Relationship between chain lengths of amylopectin and its subfraction F2 (see Fig. 3).

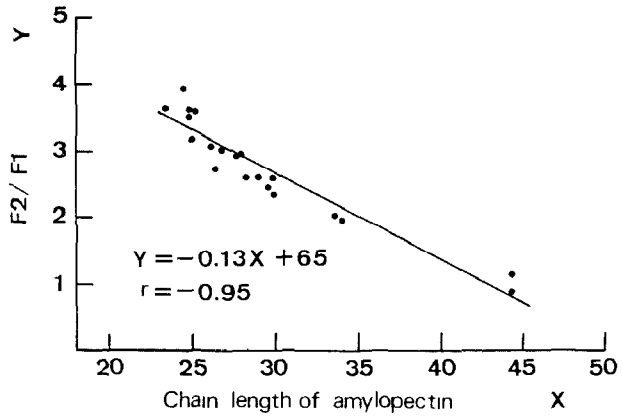



Fig. 5. Relationship between weight ratio of subfractions (F2/F1) and chain lengths of amylopectin (see Fig. 3).

that amylopectins with chain lengths in this range possibly yield any type of crystalline structure affected by the growth temperature[2,3] or by lipid or other materials[29,30]. In fact, it has been observed that the C-type starches of sweet potato and soybean change as to their crystalline structures, depending on the environmental temperature[2,3]. However, no such temperature-dependent transitions have been found in the A-type starches of rice[31] and Chlorella[32] or the B-type starch of potato[31]. Thus, the sensitivity to temperature differs from species to species. Together with the previous findings, the present results suggest that the sensitivity is dependent on the chain length of the amylopectin. The A- and B-type starches with relatively short and long chains, respectively, are less sensitive to temperature than the C-type starches with intermediate chain-lengths, and they maintain their own crystalline structures even if they are subjected to a relatively large change

(15°) in temperature. The characteristic structure of amylopectin by species is considered to depend on the activities and specificities of the enzymes involved in the biosynthesis of the molecule. In fact, several forms of starch synthase and branching enzyme, with different substrate specificities, have been found in some plants[33]. Therefore, it may be concluded that the chain length of amylopectin is an intrinsic factor that determines the crystalline structure of starch granules.

The structural implication of the bimodal distribution of the long and short chains is not yet clear, but it may be inferred that the long- and short-chain fractions are mainly long B-, and the short B- and A-chains[26], respectively[7,34]. The F2:F1 ratios on a molar basis, which were calculated from the ratios on a weight basis and the weight-average chain-lengths, were 8–12 for the A-type, 3–7 for the B-type, and 7–9 for the C-type starches (Table II). The trimodal distribution of some specimens suggests that the F2 chains may be composed of two populations, short B-chains and A-chains, with close distributions.

The bimodal or trimodal distribution of chains is an important feature for discriminating crystalline amylopectin from amorphous glycogen molecules. Glycogen may be regarded as a limit form of amylopectin with no F1 chains. The length of the F1 chain is closely correlated with that of the whole amylopectin. The F1 chain may direct the formation of the crystalline polymorphs by playing the role of a nucleus in the crystallisation.

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