Carbohydrate Research, 157 (1986) 215–234
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

QUANTITATIVE ASPECTS OF AMYLOSE–LIPID INTERACTIONS

JOHN KARKALAS AND STILIANOS RAPHAELIDES

Food Science Division, Department of Bioscience and Biotechnology, University of Strathclyde, 131 Albion Street, Glasgow G1 1SD (Great Britain)

(Received April 29th, 1986; accepted for publiation, July 14th, 1986)

ABSTRACT

The formation of complexes between amylose and the potassium salt of fatty acids in 0.01M KOH (pH 12) was studied by capillary viscometry at 20°. The viscosity number of the clear solutions decreased with increasing concentration of fatty acids (12:0 to 22:0 with an even number of carbon atoms) and reached a constant value presumably because of saturation of the amylose helix. At saturation, the molar ratio of fatty acid/amylose was dependent on the chain length of the fatty acid; the longer the chain length the lower the saturation molar ratio. Insoluble amylose–fatty acid complexes were obtained after neutralisation of the alkaline solutions and adjustment of the pH to 4.6. Essentially the same saturation molar ratios were found by determination of the precipitated complexed amylose. Fatty acids were not readily extractable with diethyl ether from the precipitated complexes and their extractability decreased with increasing molecular weight. Unsaturated fatty acids, with 1–4 double-bonds (all *cis*), were slightly less effective in forming complexes than their saturated counterparts, apparently because of steric constraints. Caprylic (8:0) and capric acids (10:0) did not form complexes at pH 12, although they did form insoluble complexes at pH 4.6. Monoglycerides of fatty acids, as liposomes, readily formed insoluble complexes at pH 4.6. Experimental techniques for the determination of the complexing ability of fatty acids and their monoglycerides in model systems are presented.

INTRODUCTION

The interaction of amylose, the essentially linear component of starch, with various organic molecules to give insoluble complexes has been the subject of numerous investigations. It is now well established that unbranched polar compounds, *e.g.*, fatty acids and their monoglycerides, form helical inclusion complexes with amylose under suitable conditions[1–4]. These complexes (clathrates) are considered to be similar to the blue polyiodide complex of amylose whose structure had been originally elucidated by Rundle and Baldwin[5]. The complexed or guest molecule is thought to occupy the central axis of a helix consisting of 6, 7, or 8 glucosyl residues per turn, with a repeat spacing (pitch) of 0.8 nm. The complexes

0008-6215/86/$ 03.50

have been extensively studied by X-ray diffraction crystallography[6–8] and give rise to the so-called V-pattern of amylose.

More recently, differential scanning calorimetry (d.s.c.) has been used for the measurement of the dissociation temperature and enthalpy of the complexes[9–13]. Relatively little work has been done on the solution properties of the complexes[14] and particularly on the quantitative relations of fatty acids and amylose[4], and it is only recently that the specific rotation and surface tension of solutions of amylose–fatty acid complexes have been investigated[15,16]. The flow birefringence of amylose–iodine complexes and the lowering of the viscosity number of amylose–1-butanol complexes have been cited by Banks and Greenwood[17] as unequivocal evidence for the helical structure of the complexes. The same authors strongly opposed the view that amylose assumes a helical structure in solution in the absence of guest molecules. Moreover, they stated that the solvation requirements of the helical cavity are such that the helix could not exist in aqueous solution in the absence of a complexing agent. It is thought that the interaction of the complexing agents with the wall of the helical cavity is mainly hydrophobic[18].

The formation of complexes is of technological importance; *e.g.*, in baking, the incorporation of fatty acid monoglycerides in the dough is known to retard the staling of bread[19,20]. Monoglycerides are also added to dried potato granules used for instant mash, in order to prevent stickiness[21]. A systematic investigation of the quantitative relationships of fatty acid–amylose interactions has not been reported. The present study was initiated in order to elucidate aspects of the conformational change of amylose in alkaline solution (pH 12) on the addition of fatty acids and to explore the nature and composition of amylose–fatty acid (and monoglyceride) complexes precipitated at pH 4.6 after neutralisation of the alkali.

EXPERIMENTAL

Materials. — Potato amylose (A-9262), fatty acids, and fatty acid monoglycerides (not less than 99% pure) were obtained from the Sigma Chemical Company. Amyloglucosidase (208469; activity, 6 units/mg, 75% protein content) was obtained from Boehringer–Mannheim. All other reagents were of analytical grade.

Preparation of amylose solutions. — Amylose (3 g/100 mL) was dissolved in dimethyl sulphoxide by continuous stirring for 24 h at room temperature. The solution was centrifuged for 30 min at 2000*g* in order to remove traces of suspended matter, and the amylose in the supernatant solution was precipitated by the addition of 2 volumes of 1-butanol. The precipitated amylose was recovered by centrifugation after three successive washings with 1-butanol and stored as such. Amylose was obtained as an aqueous solution on the addition of water to the butanol complex followed by heating for 60 min in a boiling water bath while passing a stream of nitrogen through the mixture. The concentration of amylose was measured by an enzymic method[22]. Its purity (>99%) was confirmed by a blue

value method[23], and its beta-amylolysis limit was 83% in accord with published values[17]. The molecular weight was estimated from the measurement of the limiting viscosity number ($[\eta]$ = 134.5 mL/g) in 0.15M KOH, according to the Mark–Houwink relation $[\eta] = 8.36 \times 10^{-3} M_w^{0.77}$, as reported by Banks and Greenwood[24]. The viscosity average molecular weight (M_w) was 2.9×10^5, corresponding to a d.p. of ~1800.

Preparation of complexes in solution. — To the aqueous solution of amylose, prepared as described in the previous section, 0.1M KOH was added immediately and diluted to 0.01M by the addition of water. The final concentration of amylose was ~15 mg/mL. The solution was filtered through a Pyrex sintered-glass filter (porosity 2) and used within 1 h. The potassium salts of fatty acids were obtained by the addition of a calculated volume of 0.1M KOH to a given mass of fatty acid followed by heating (water bath) to the melting point of the fatty acid. The resulting clear solution was diluted with 0.01M KOH to a fatty acid concentration of 1 mg/mL and used at once. Complexes were prepared in 20-mL capacity Pyrex tubes provided with screw-caps fitted with PTFE-lined rubber discs. For this purpose, 4 mL of amylose solution (~60 mg in 0.01M KOH) were transferred into each tube, followed by the addition of the required volume of fatty acid solution. The final volume was made up to 15 mL with 0.01M KOH. The well mixed samples were stored overnight at room temperature before measurement of the viscosity. A control solution containing amylose only (~4 mg/mL) remained perfectly clear and no change in the viscosity could be detected within 24 h. Most of the samples also remained clear, but a slight turbidity was observed with high levels of added fatty acids. Turbid samples were filtered through glass microfibre filters (Whatman GF/A). The determinations described in the following sections were carried out in triplicate.

Capillary viscometry. — Viscosity was measured by means of an Ubbelohde suspended-level glass viscometer specially made to accommodate a large sample volume and to give a flow time of ~190 s with the pure solvent. Reproducibility of flow times was ensured by regular cleaning with chromic–sulphuric acid mixture. The temperature was controlled at 20 ±0.1°.

Determination of amylose. — On completion of the viscosity measurements, alkaline samples (3 mL) were transferred into Pyrex tubes (20-mL capacity, fitted with PTFE-lined screw-caps) and neutralised with 0.01M HCl (3 mL). Citrate buffer (6 mL, containing 460 mg of citric acid monohydrate and 840 mg of sodium citrate dihydrate in 100 mL of water, pH 4.6) was added and the contents were mixed thoroughly by vortex-mixing. Aliquots (1 mL) were transferred immediately into screw-cap tubes (10-mL capacity) and 1 mL of amyloglucosidase solution (2 mg/mL of citrate buffer = 12 units) was added. After hydrolysis at 60° for 30 min, 8 mL of water were added and the glucose was determined by a glucose oxidase method[22]. The remaining samples were allowed to stand at room temperature overnight and then centrifuged at 2000*g* for 15 min. The supernatant solution was filtered through a Millipore filter (RAWPO1300; pore size, 1.2 μm), and the residual amylose in the filtrate was determined as described earlier.

Amylose–monoglyceride complexes. — Alkaline degradation of monoglycerides of fatty acids was avoided by the preparation of liposomes in an aqueous medium[25]. A 10% dispersion of monoglyceride was prepared by the addition of aqueous 1% potassium cholate. The dispersion was heated to 60° for 15 min and sonicated for 5 min. The translucent and homogeneous dispersion was added to a freshly prepared aqueous solution of amylose, vortex-mixed, and heated to 60° for 30 min, with the exception of the mono-oleate which was heated to 40°. After precipitation overnight, the complexes were removed by centrifugation and the filtered supernatant solutions were used for the determination of residual amylose.

Gas–liquid chromatography. — Solutions of amylose and fatty acid were prepared in 0.01M KOH. For complex formation, 1 mL of amylose solution was added to 1 mL of fatty acid solution and vortex-mixed in a Pyrex tube (10-mL capacity, fitted with a PTFE-lined screw-cap). The concentration of amylose was ~13 mg/mL, while that of the fatty acids varied in the range 0.1 to 1.2 mg/mL according to the molecular weight. Capric (10:0), lauric (12:0), palmitic (16:0), stearic (18:0), and arachidic (20:0) acids were used, each at five different concentrations. Complexes were prepared in duplicate for each fatty acid concentration. A third tube containing fatty acid and 1 mL of 0.01M KOH, but no amylose, served as a control. The mixtures were heated in a water bath at 60° for 30 min, cooled, neutralised with 2 mL of 0.01M HCl, adjusted to pH 4.6 with 1 mL of citrate buffer, and left at room temperature overnight. One of the replicates was treated with a solution of amyloglucosidase (2 mg/mL ≡ 12 units) in citrate buffer at 60° for 2 h to hydrolyse the amylose completely and to release the fatty acid. Subsequently, the tubes containing the control, the complex, and the amyloglucosidase-hydrolysed sample, for each level of added fatty acid, were used for the extraction of the fatty acid with diethyl ether (3 × 2 mL). To the ether extracts was added heptadecanoic (17:0) acid (~0.4 mg) as an internal standard, and the ether was evaporated to dryness in a stream of nitrogen. The methyl esters of the fatty acids were prepared by the addition of 1 mL of methanol–boron trifluoride[26]. Fatty acid methyl esters were separated on a glass column (2.1 m × 2 mm i.d.) packed with 15% of EGSSX on Gaschrom P (100–120 mesh) or 15% of CP-Sil 84 on Chromosorb WHP (100–120 mesh). A Pye 104 gas chromatograph was used with a flame-ionisation detector. The injector, oven, and detector temperature was 185°. The carrier gas (nitrogen) and the hydrogen flow-rates were 30 mL/min, and the air flow-rate was 500 mL/min.

RESULTS

Viscometric measurements. — The viscosity number (*i.e.*, the specific viscosity divided by the concentration of the polymer in g/mL) of amylose in solution (pH 12, at 20°) as a function of the molar ratio (mol of fatty acid/mol of amylose, FA/AM) is shown in Fig. 1. The molecular weight of amylose was taken as that (162) of the "anhydroglucose" monomer ($C_6H_{10}O_5$). A sharp decrease in

the viscosity number was observed on the addition of increasing quantities of fatty acids as potassium salts. With myristic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0), and behenic (22:0) acids, an almost constant viscosity was reached and this suggests that the amylose helix was saturated with the fatty acid anions. Saturation occurred at a lower molar ratio as the chain length of the fatty acid increased. With lauric (12:0) acid, saturation was not attained, probably because of electrostatic repulsion of the short-chain anions inside the helical cavity of amylose. The viscosity numbers for oleic acid (*cis*-unsaturated) and elaidic acid (*trans*-unsaturated) complexes are shown in Fig. 2. Elaidic acid was more effective than oleic acid at a high molar ratio, probably because of the quasi-linearity of the molecule. The effect of other unsaturated fatty acids is shown in Figs. 3 and 4. All *cis*-unsaturated acids showed good complexing ability and arachidonic acid, with 4

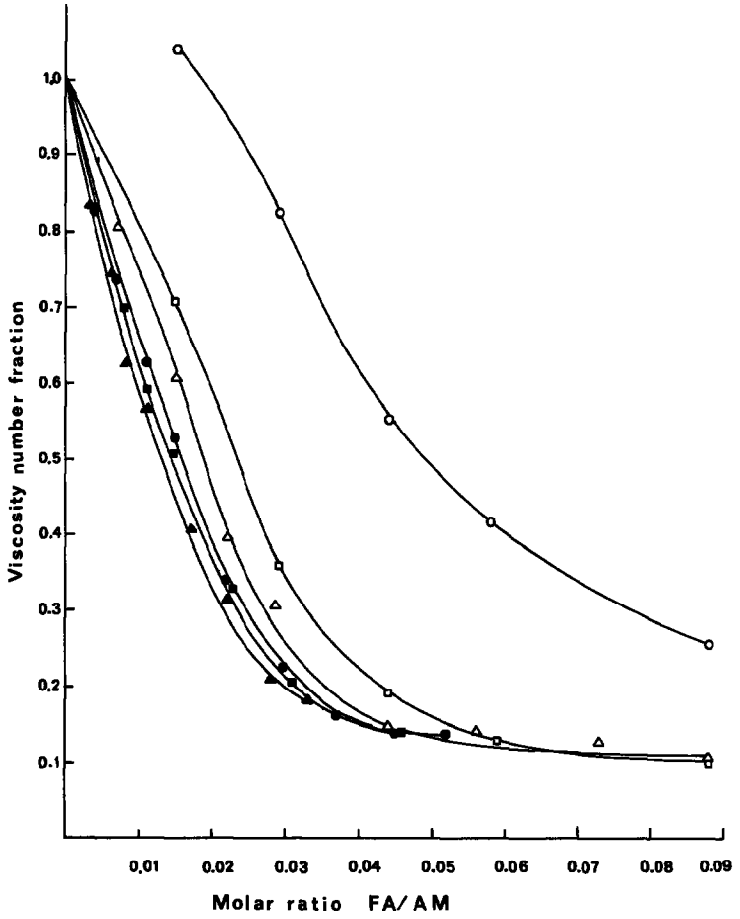

Fig. 1. Viscosity number of amylose (4 mg/mL) in 0.01M KOH (pH 12.0) with added fatty acid potassium salt: ○, lauric; □, myristic; △, palmitic; ●, stearic; ■, arachidic; ▲, behenic.

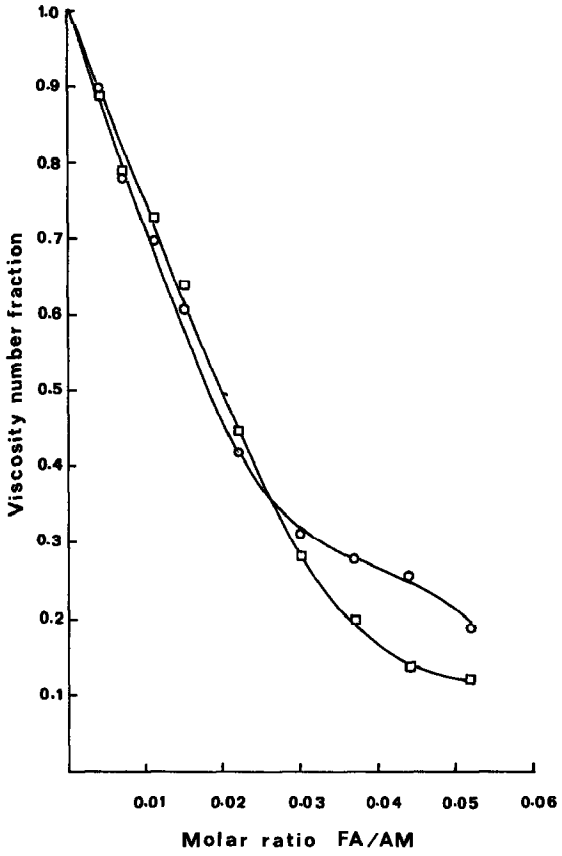

Fig. 2. Viscosity number as in Fig. 1 with ○, oleic (*cis*); □, elaidic (*trans*).

cis-double bonds, showed only a small difference from its saturated counterpart. It is emphasised that the foregoing measurements were carried out at pH 12 with solutions that were visually perfectly clear.

Precipitation of amylose–lipid complexes. — Insoluble precipitates of the complexes were obtained after neutralisation of the alkaline solutions used for viscosity measurements and adjustment of the pH to 4.6. Precipitated, complexed amylose was calculated after determination of the residual amylose as described earlier. As shown in Figs. 5–8, at least 98% of the amylose formed a complex. Similar results were obtained with the monoglycerides of fatty acids (Fig. 9). Capric (10:0) acid did not appear to form a complex at pH 12, but gave complete precipitation of amylose at pH 4.6 (Fig. 10). Caprylic (8:0) acid gave rise to complete precipitation at a high molar ratio as expected, whereas butyric (4:0) acid did not give a precipitate even at very high levels.

Gas–liquid chromatography. — Fatty acids were determined by g.l.c. after extraction with diethyl ether (see Experimental). The results are shown in Table I.

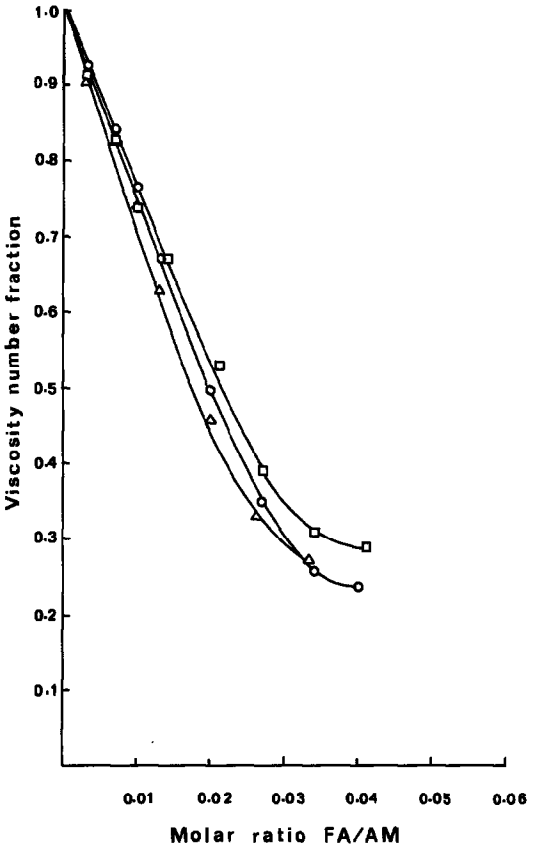

Fig. 3. Viscosity number as in Fig. 1 with ○, linolenic; □, linoleic; △, eicosenoic.

Complexed fatty acids were calculated as the difference between added fatty acids (control) and ether-extractable fatty acids (free). After hydrolysis (with amyloglucosidase), it was expected that the extracted fatty acids would be equal to the control. The incomplete recovery after hydrolysis is a reflection of the difficulty in extracting the fatty acids from the aqueous medium, due to proteinaceous material contributed by the enzyme because high levels of amyloglucosidase (*e.g.*, 10 mg/mL ≡ 7.5 mg of protein ≡ 60 units) resulted in low recoveries. It is possible that a fatty acid–protein interaction at the ether/water interface prevented the complete extraction of the acid. Replicate determinations of amylose[22] indicated that virtually complete hydrolysis of amylose–lipid complexes was achieved by the method employed. Thus, incomplete enzymic degradation of the complexes cannot explain the low recovery figures. An attempt to use carbon tetrachloride as the solvent gave rise to an emulsion and its use was discontinued. The results in Table I also suggest that complexed fatty acids with a long chain (16:0, 18:0, and 20:0) were protected within the helix and, therefore, could not be readily extracted

TABLE I

FATTY ACIDS EXTRACTED WITH DIETHYL ETHER FROM AMYLOSE COMPLEXES BEFORE AND AFTER HYDROLYSIS WITH AMYLOGLUCOSIDASE

Control[a]	*Ether-extractable (free)*	*Complexed (by difference)*	*Extracted after hydrolysis*[b]	*Recovery per cent by hydrolysis*	*Molar ratio*[c]
Capric acid (mg)					
0.163	0.052	0.111	0.135	83	0.008
0.334	0.123	0.211	0.305	90	0.014
0.564	0.158	0.406	0.493	87	0.028
0.746	0.368	0.378	0.661	89	0.026
1.173	0.892	0.281	0.970	83	0.019
Lauric acid (mg)					
0.213	0.000	0.213	0.106	50	
0.390	0.000	0.390	0.186	48	0.024
0.580	0.022	0.558	0.360	61	0.034
0.780	0.043	0.737	0.451	58	0.045
1.170	0.316	0.854	0.920	79	0.052
Palmitic acid (mg)					
0.200	0.000	0.200	0.140	70	
0.408	0.000	0.408	0.292	72	
0.593	0.000	0.593	0.416	70	
0.790	0.000	0.790	0.556	70	0.037
1.152	0.330	0.820	1.040	90	0.038
Stearic acid (mg)					
0.132	0.000	0.132	0.095	72	
0.278	0.000	0.278	0.212	76	
0.476	0.000	0.476	0.321	67	
0.563	0.000	0.563	0.295	52	0.024
0.944	0.065	0.879	0.481	51	0.038
Arachidic acid (mg)					
0.105	0.000	0.105	0.050	48	
0.250	0.000	0.250	0.154	62	
0.410	0.000	0.410	0.274	67	
0.476	0.000	0.476	0.347	73	0.021
0.747	0.036	0.711	0.672	90	0.036

[a]Added fatty acid. [b]Includes free and complexed fatty acid. [c]Complexed fatty acid/amylose.

with diethyl ether. However, small quantities of these acids were extracted as saturation of the helix was approached. It is also noteworthy that short-chain fatty acids (10:0 and 12:0) were easily extracted from the helix and this is in accord with their non-ideal behaviour as explained in the Discussion.

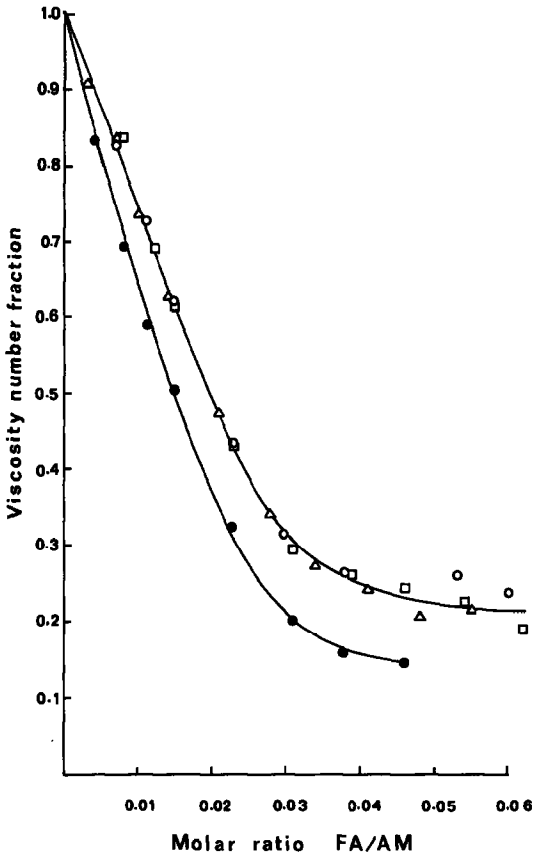

Fig. 4. Viscosity number as in Fig. 1 with ○, eicosadienoic (all *cis*); □, eicosatrienoic (all *cis*); △, arachidonic (all *cis*); ●, arachidic.

DISCUSSION

The conformation of amylose in solution has been a controversial matter for many years[27]. The hydrodynamic behaviour of amylose in dilute solution was studied by Banks and Greenwood[14] who provided evidence that amylose in neutral aqueous solution possesses no helical character. The conformation of amylose in other solvent systems has been summarised by the same authors as follows: random coil in water and in neutral aqueous KCl; expanded coil in formamide, in Me_2SO, and in aqueous alkali; helical in neutral solution + complexing agent, in alkaline solution + complexing agent, and in aqueous solution (pH 12) in the presence of 0.3M KCl.

From X-ray diffraction studies of crystalline complexes of amylose with a variety of organic molecules, it is known that each turn of the amylose helix may

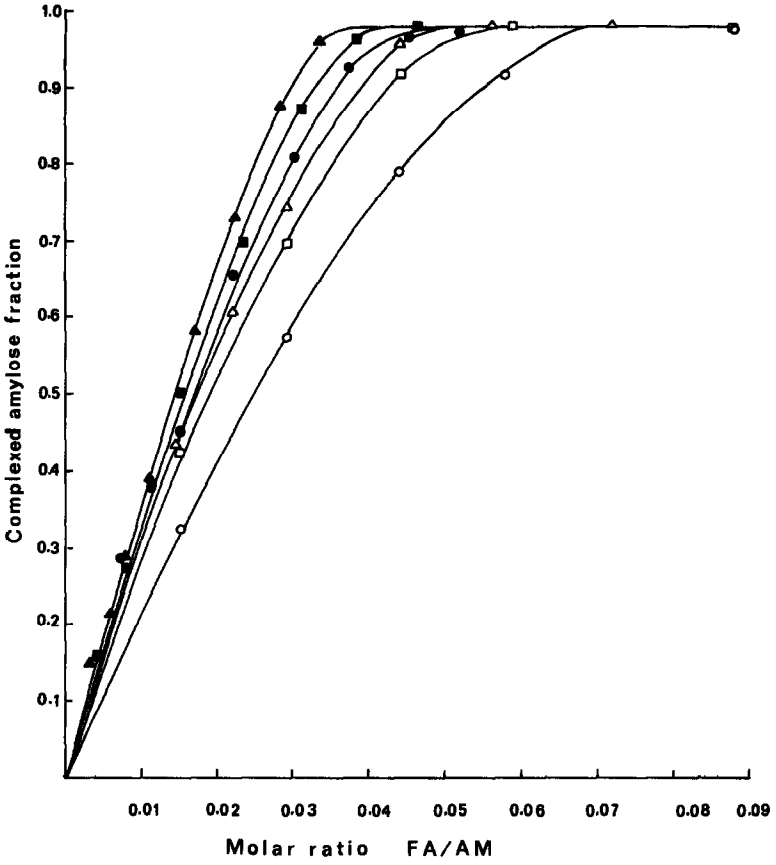

Fig. 5. Complexed amylose precipitated at pH 4.6 with ○, lauric; □, myristic; △, palmitic; ●, stearic; ■, arachidic; ▲, behenic acid.

consist of 6, 7, or 8 glucosyl residues[28–30] and that the number of residues depends on the size and shape of the guest molecule[18,28,31–33] as well as the degree of hydration of the complex[34,35]. The majority of the authors appear to agree that the repeating distance, or pitch, between adjacent helical turns of the same coil is very nearly 0.8 nm.

In the present work, the chain length of fatty acids was calculated from published data[36,37] for bond lengths and bond angles. The calculated values in Table II (column 3) are in good agreement with X-ray diffraction data[38]. For the non-ionised acids, assuming no gaps between adjoining molecules, the number of glucosyl residues per single fatty acid was also calculated. Little is known regarding the exact arrangement of the fatty acids inside the amylose helix. It is possible that, at pH 4.6, the acid molecules occupy the central cavity as paired dimers linked by hydrogen bonding at their carboxylic ends, and that the aliphatic chains are

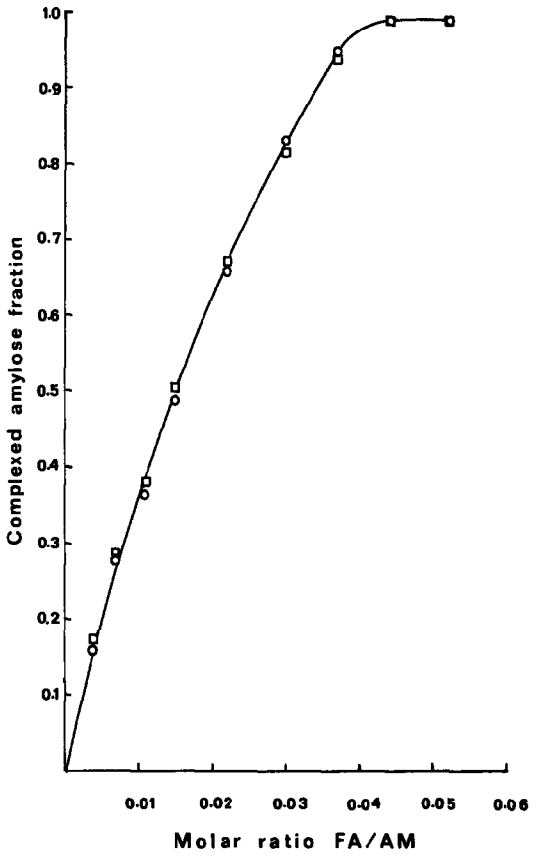

Fig. 6. Complexed amylose precipitated at pH 4.6 with ○, oleic; □, elaidic acid.

TABLE II

CALCULATED FATTY ACID CHAIN-LENGTH, NUMBER OF GLUCOSYL RESIDUES PER FATTY ACID, AND SATURATION MOLAR RATIO OF FATTY ACID/AMYLOSE

Fatty acid	*Carbon atoms*	*Length (nm)*	*Glucosyl residues per fatty acid*			*Molar ratio (FA/AM)*		
			6[a]	*7*[a]	*8*[a]	*6*[a]	*7*[a]	*8*[a]
Capric	10	1.48	11.1	13.0	14.8	0.090	0.077	0.068
Lauric	12	1.73	13.0	15.1	17.3	0.077	0.066	0.058
Myristic	14	1.98	14.9	17.3	19.8	0.067	0.058	0.050
Palmitic	16	2.23	16.7	19.5	22.3	0.060	0.051	0.045
Stearic	18	2.49	18.7	21.8	24.9	0.054	0.046	0.040
Arachidic	20	2.74	20.6	24.0	27.4	0.049	0.042	0.037
Behenic	22	2.99	22.4	26.2	29.9	0.045	0.038	0.033

[a]Numbers correspond to 6, 7, and 8 glucosyl residues per helical turn, with a repeating distance (pitch) of 0.8 nm between turns.

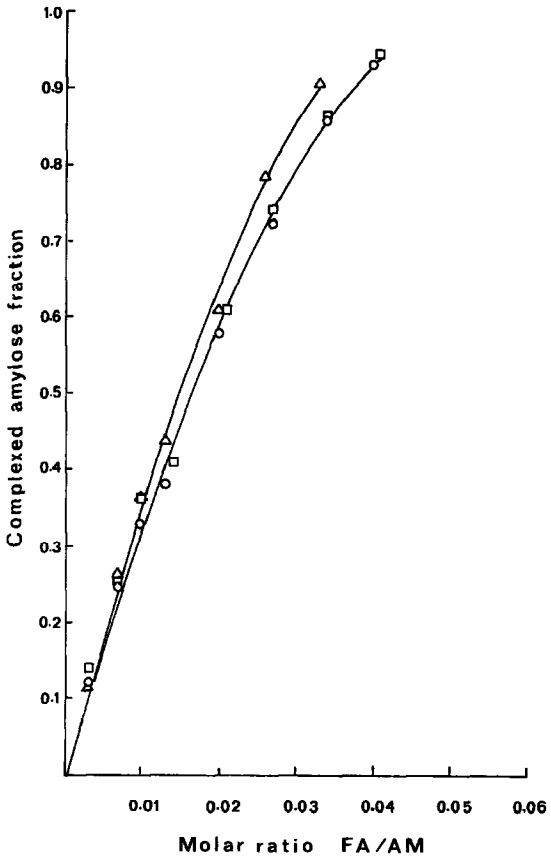

Fig. 7. Complexed amylose precipitated at pH 4.6 with ○, linolenic; □, linoleic; △, eicosenoic acid

stabilised by hydrophobic interactions in the interior of the helix. However, this arrangement may not prevail at pH 12 because of ionisation of the fatty acid salts. Table II also gives the number of glucosyl residues for each fatty acid chain and the corresponding saturation molar ratio of fatty acid/amylose (FA/AM). The structures of palmitic acid and 1-*O*-palmitoylglycerol are shown in Fig. 11, together with information for the calculation of the chain length. It is noteworthy that, since the minimum number of glucosyl residues per helical turn is known to be 6, the highest possible molar ratio (FA/AM) is as shown in Table II for various fatty acids. It is, of course, assumed that the helical cavity is saturated by the linearly arranged fatty acid molecules and that no fatty acid molecules occupy the interstitial spaces between the folding lengths of the helices on the formation of crystalline complexes. For stearic acid, for instance, 9.5 mg of acid are requied to saturate 100 mg of amylose. A higher figure would suggest accumulation of fatty acid in the interstices.

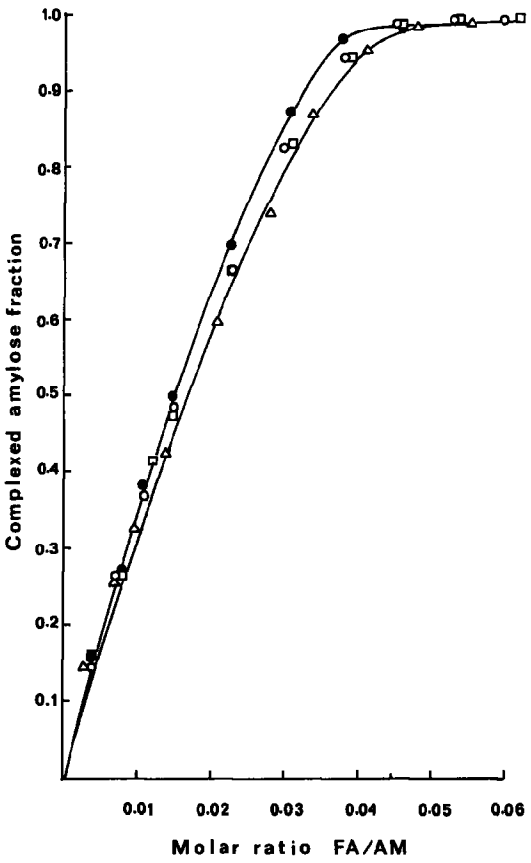

Fig. 8. Complexed amylose precipitated at pH 4.6 with ○, eicosadienoic; □, eicosatrienoic; △, arachidonic; ●, arachidic acid.

The results for the viscosity number of amylose with added fatty acids at pH 12 (Fig. 1) imply that the amylose helix is saturated by the complexed fatty acid anions, since there is no further decrease in the viscosity number on the addition of excess of fatty acid. The same effect is seen even more clearly at pH 4.6 (Fig. 5) when the insoluble complexes are precipitated from solution. Table III shows that, for each fatty acid complexed at pH 12 and at pH 4.6, saturation of the amylose helix occurs at a molar ratio FA/AM which, within experimental error, is in good agreement with that calculated on the assumption that there are 7 glucosyl residues per turn. However, the X-ray diffraction data for amylose–fatty acid complexes of Takeo *et al.*[28] suggest that each helix consists of 6 glucosyl residues for acetic (2:0), caproic (6:0), caprylic (8:0), and higher fatty acids, while propionic (3:0), butyric (4:0), and valeric (5:0) acids form helices with 7 glucosyl residues. In the light of the present results, it can be assumed that, for six-membered helices, the fatty acids

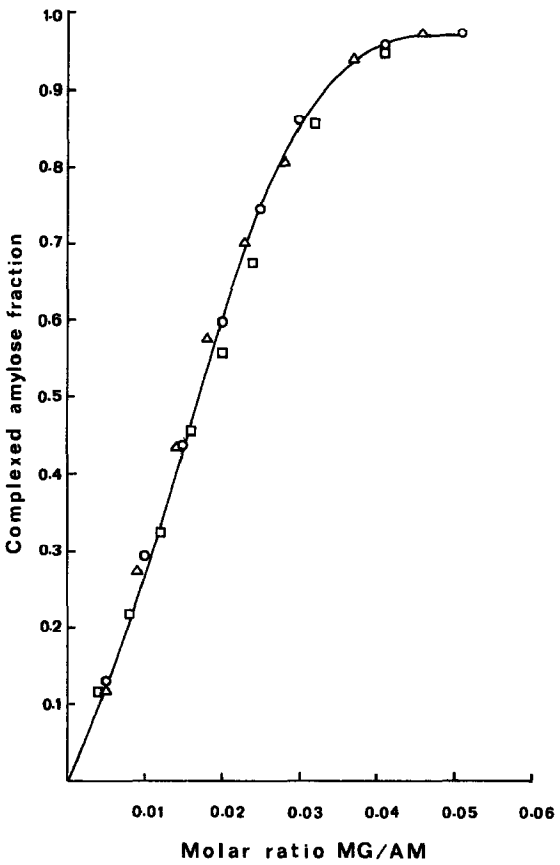

Fig. 9. Complexed amylose precipitated at pH 4.6 with liposomes of ○, 1-*O*-palmitoyl-*rac*-glycerol; □, 1-*O*-stearoyl-*rac*-glycerol; △, 1-*O*-oleoyl-*rac*-glycerol.

in the central axis of the saturated clathrate interact with 6/7 or 86% of the available glucosyl residues. Recent work by Jane and Robyt[33] supports earlier findings[31,32] that complexed amylose forms lamellar crystals in which the helical chains are oriented perpendicular to the lamellae with a thickness, or folding length, of 10 nm. These authors proposed that each folding end consists of an amorphous region which is susceptible to attack by alpha-amylase and provided evidence that for a six-membered helix, with a pitch of 0.8 nm, there are 75 glucosyl residues per folding length of 10 nm. It is conceivable that each amorphous folding-end consists of ~12 glucosyl residues which do not enclose a fatty acid molecule, and thus 75/87 of the glucosyl residues (~86%) participate in complex formation as shown in the present work. It is also possible that the fatty acid molecules inside the six-membered helix are evenly spaced and separated by short gaps which correspond to 1/7 of the total length of the complexed amylose chain.

Recently, Bulpin *et al.*[16] provided evidence for saturation of the amylose helix

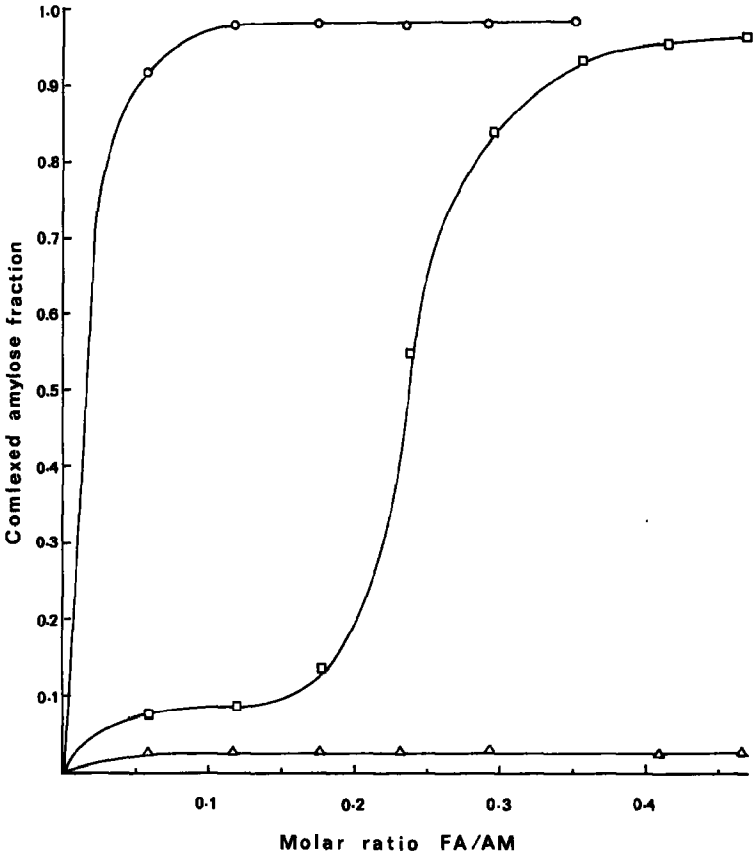

Fig. 10. Complexed amylose precipitated at pH 4.6 with ○. capric; □, caprylic; △, butyric acid.

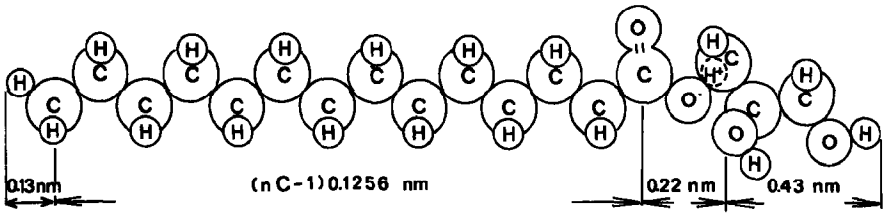

Fig. 11. Model structure of palmitic acid and 1-*O*-palmitoyl-*rac*-glycerol. Hydrocarbon chain-length = (nC − 1)0.154cos35.35° = (nC − 1)0.1256. Total length of non-ionised fatty acid: L = (nC − 1)0.1256 + 0.13 + 0.22 nm. Length of 1-*O*-palmitoylglycerol: L = (nC − 1)0.1256 + 0.13 + 0.22 + 0.43 nm.

TABLE III

EXPERIMENTAL MOLAR RATIOS OF LIPID/AMYLOSE FOR SATURATED AMYLOSE HELICES AND CALCULATED VALUES BASED ON THE LIPID CHAIN-LENGTH

Lipid	*pH 12*[a]	*pH 4.6*[b]	*G.l.c.*[c]	*Calculated*[d]
Capric	—	0.100	0.029	0.077
Lauric	—	0.068	0.052	0.066
Myristic	0.060	0.058		0.058
Palmitic	0.055	0.052	0.038	0.051
Stearic	0.046	0.047	0.038	0.046
Arachidic	0.042	0.042	0.036	0.042
Behenic	0.040	0.038		0.038
Oleic (*cis*)	0.050	0.044		0.046
Elaidic (*trans*)	0.048	0.044		0.046
Linoleic (*cis*)	0.041	0.044		
Linolenic (all *cis*)	0.041	0.044		
Eicosenoic (*cis*)	—	0.042		
Eicosadienoic (all *cis*)	0.048	0.045		
Eicosatrienoic (all *cis*)	0.048	0.045		
Arachidonic (all *cis*)	0.048	0.048		
Monopalmitoylglycerol	—	0.046		0.043
Monostearoylglycerol	—	0.046		0.039
Mono-oleoylglycerol	—	0.046		

[a]Capillary viscometry data. [b]By determination of precipitated amylose. [c]By extraction with diethyl ether and g.l.c. [d]Molar ratio for assumed 7 glucosyl residues per helical turn.

by sodium myristate and longer-chain fatty acids, based on measurements of optical rotation, n.m.r., and surface tension. They also found, in agreement with the present work, that short-chain fatty acids (below 10:0) did not give rise to saturation. The authors attributed this to adsorption of the acids onto the amylose. We propose that incomplete complex formation is due to electrostatic repulsion of the short-chain fatty acid anions and possibly to the absence of adequate hydrophobicity. Whittam *et al.*[39] reported that the addition of sodium caprate to a neutral aqueous solution of amylose caused a conformational transition over the range of lipid:amylose ratios from 0.2:1 to 1:1 by weight, and that above a ratio of 1:1, further addition of lipid had no effect. Our inability to obtain an insoluble complex with butyric acid can be attributed to the low concentration of reagents used, although Bulpin *et al.*[16] failed to observe complex formation even at saturating concentrations of sodium butyrate. By contrast, several methods reported in the literature make use of a comparatively high concentration of amylose and a large excess of complexing agents[28,33,39]. None of the methods appears to take into account the possible existence of a quantitative relationship between the guest molecule and amylose.

The terms "amylose complexing index" and "relative complexing efficiency" have been used by Krog[20] and by Riisom *et al.*[25] to characterise the interaction of

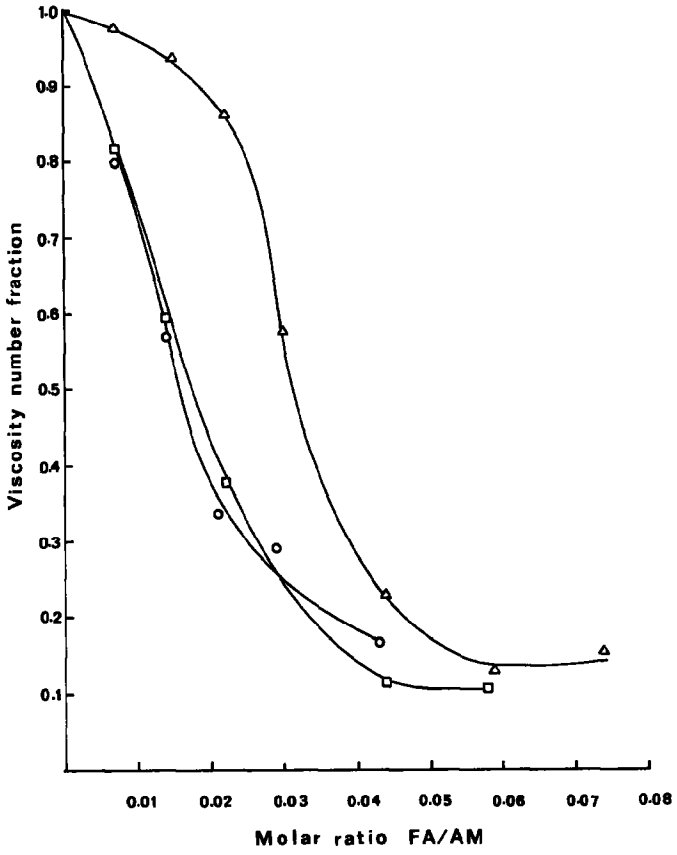

Fig. 12. Viscosity number of amylose–palmitic acid in ○, 0.001M KOH (pH 10.7); □, 0.01M KOH (pH 12.0), △, 0.1M KOH (pH 13.1).

saturated and unsaturated monoglycerides with amylose. In order to eliminate the effects of different mesomorphic states, the monoglycerides were used by the latter authors in the form of liposomes[25], as in the present work. Our results (Fig. 9) show that, within experimental error, there is no difference between the complexing ability of monostearate, monopalmitate, and mono-oleate, and that the saturation molar ratio is ~0.046. Recalculation of the data of Riisom *et al.*[25] for monopalmitate and mono-oleate gives molar ratios of 0.033 and 0.024, respectively, and therefore incomplete filling of the helix is indicated. The direct measurement of amylose by the enzymic method[22] simplifies the procedure considerably and the use of molar ratios obviates the need for the definition of arbitrary quantities.

The effect of the concentration of KOH on the viscosity number of amylose solutions on the addition of palmitic acid is shown in Fig. 12. The conformational

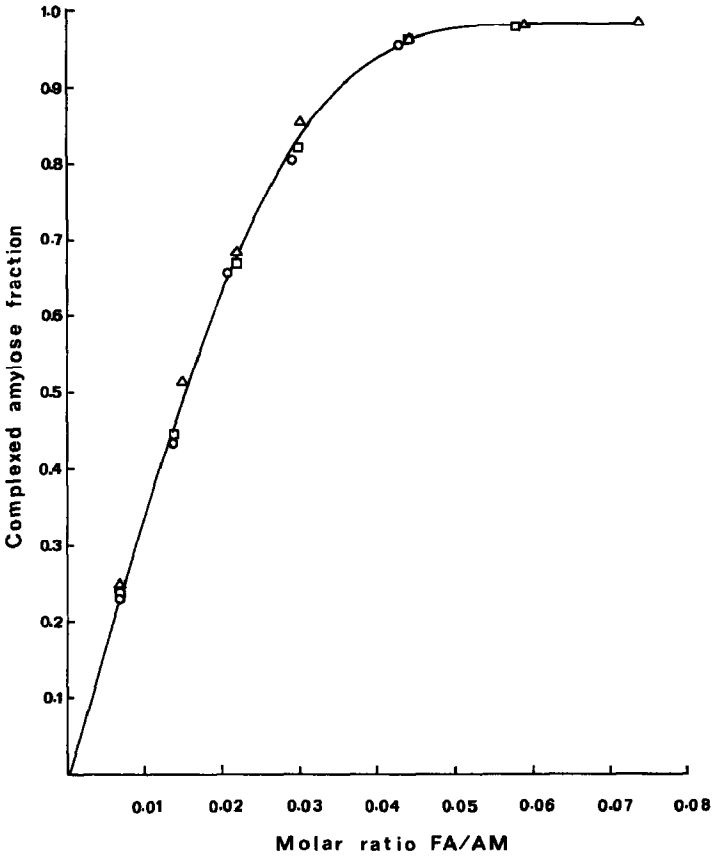

Fig. 13. Complexed amylose precipitated at pH 4.6 with palmitic acid from original KOH solutions: ○, 0.001M; □, 0.01M; △, 0.1M.

transition occurred at a higher molar ratio for 0.1M KOH (pH 13.1), compared with 0.01M (pH 12.0) or 0.001M KOH (pH 10.7), and this suggests that the amylose is, in part, in the form of an expanded coil at a very high pH. At this high pH value, the amylose possesses polyelectrolyte character, the induced negative charges repel one another, and the coil dimensions, and thus the limiting viscosity number, increase[17]. In contrast, after neutralisation and precipitation of the complexes at pH 4.6, saturation of the helix occurred at exactly the same molar ratio, regardless of the original pH, as shown in Fig. 13.

The effect of temperature on the viscosity number is shown in Fig. 14. At 65°, the palmitate complex did not show signs of dissociation even at pH 12. The dissociation temperature of the palmitate complex has been variously reported as 90[15], 95[39], 103[40], and 109°[12].

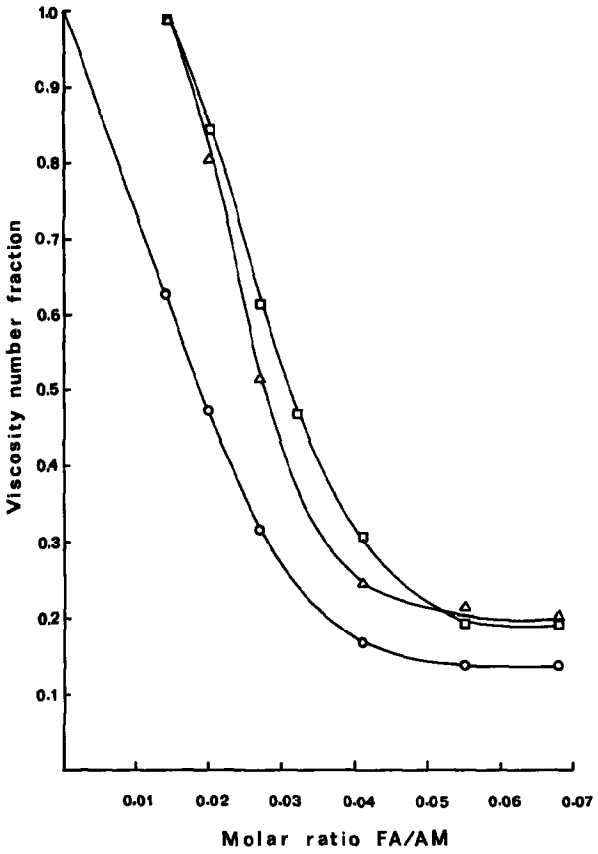

Fig. 14. Viscosity number of amylose–palmitic acid in 0.01M KOH: ○, complexed at 20° and viscosity measured at 20°; □, complexed at 20° and measured at 65°; △, complexed at 65° and measured at 65°.

CONCLUSIONS

The results of the present work show that the formation of complexes between fatty acids and amylose, in model systems, involves saturation of the amylose helix and that this occurs at a molar ratio (FA/AM) which can be predicted with reasonable accuracy from the chain length of the fatty acid molecules. About 6/7 or 86% of the glucosyl residues of the amylose appear to interact with the fatty acids under the experimental conditions used. The amylose helix can accommodate irregularities in the lipid molecule, *e.g.*, double bonds or 1-glycerol ester bonds. *cis*-Unsaturated fatty acids, in spite of their non-linearity, readily form complexes. Fatty acids with 10 carbon atoms, or less, do not appear to complex at pH 12, most probably because of repulsion due to the negative charge of the anions, diminished hydrophobicity, and ionisation of the amylose molecule.

ACKNOWLEDGMENT

We thank Professor W. R. Morrison, Food Science Division, University of Strathclyde, for advice during the preparation of the manuscript.

REFERENCES

1 R. E. RUNDLE AND F. C. EDWARDS, *J. Am. Chem. Soc.*, 65 (1943) 2200–2203.
2 T. J. SCHOCH AND C. B. WILLIAMS, *J. Am. Chem. Soc.*, 66 (1944) 1232–1233.
3 R. S. BEAR, *J. Am. Chem. Soc.*, 66 (1944) 2122–2123.
4 F. F. MIKUS, R. M. HIXON, AND R. E. RUNDLE, *J. Am. Chem. Soc.*, 68 (1946) 1115–1123.
5 R. E. RUNDLE AND R. BALDWIN, *J. Am. Chem. Soc.*, 65 (1943) 554–558.
6 W. T. WINTER AND A. SARKO, *Biopolymers*, 13 (1974) 1447–1460.
7 W. T. WINTER AND A. SARKO, *Biopolymers*, 13 (1974) 1461–1482.
8 V. G. MURPHY, B. ZASLOW, AND A. D. FRENCH, *Biopolymers*, 14 (1975) 1487–1501.
9 M. KUGIMIYA, J. W. DONOVAN, AND R. Y. WONG, *Staerke*, 32 (1980) 265–270.
10 M. KUGIMIYA AND J. W. DONOVAN, *J. Food Sci.*, 46 (1981) 765–770.
11 A.-C. ELIASSON AND N. KROG, *J. Cereal Sci.*, 3 (1985) 239–248.
12 W. R. MORRISON, in R. D. HILL AND L. MUNCK (Eds.), *New Approaches to Research on Cereal Carbohydrates*, Elsevier Science Publishers, Amsterdam, 1985, pp. 61–70.
13 C. G. BILIADERIS, C. M. PAGE, L. SLADE, AND R. R. SIRETT, *Carbohydr. Polym.*, 5 (1985) 367–389.
14 W. BANKS AND C. T. GREENWOOD, *Staerke*, 23 (1971) 300–314.
15 P. V. BULPIN, E. J. WELSH, AND E. R. MORRIS, *Staerke*, 34 (1982) 335–339.
16 P. V. BULPIN, A. N. CUTLER, AND A. LIPS, in G. O. PHILLIPS, D. J. WEDLOCK, AND P. A. WILLIAMS (Eds.), *Gums and Stabilisers for the Food Industry 3*, Elsevier Applied Science Publishers, London, 1986, pp. 221–232.
17 W. BANKS AND C. T. GREENWOOD, *Starch and its Components*, Edinburgh University Press, 1975, pp. 169, 173, 17, and 175.
18 D. FRENCH, A. O. PULLEY, AND W. J. WHELAN, *Staerke*, 15 (1963) 349–354.
19 N. KROG AND B. NYBO JENSEN, *J. Food Technol.*, 5 (1970) 77–87.
20 N. KROG, *Staerke*, 23 (1971) 206–210.
21 R. HOOVER AND D. HADJIYEV, *Staerke*, 33 (1981) 290–300.
22 J. KARKALAS, *J. Sci. Food Agric.*, 36 (1985) 1019–1027.
23 W. R. MORRISON AND B. LAIGNELET, *J. Cereal Sci.*, 1 (1983) 9–20.
24 W. BANKS AND C. T. GREENWOOD, *Carbohydr. Res.*, 7 (1968) 414–420.
25 T. RIISOM, N. KROG, AND J. ERIKSEN, *J. Cereal Sci.*, 2 (1984) 105–118.
26 W. R. MORRISON AND L. M. SMITH, *J. Lipid Res.*, 5 (1964) 600–608.
27 J. SZEJTLI AND S. AUGUSTAT, *Staerke*, 18 (1966) 38–52.
28 K. TAKEO, A. TOKUMURA, AND T. KUGE, *Staerke*, 25 (1973) 357–362.
29 A. D. FRENCH AND V. G. MURPHY, *Cereal Foods World*, 22 (No. 2) (1977) 61–70.
30 D. FRENCH, in R. L. WHISTLER, J. N. BEMILLER, AND E. F. PASCHALL (Eds.), *Starch Chemistry and Technology*, 2nd edn., Academic Press, Orlando, FL, 1984, pp. 200–208.
31 Y. YAMASHITA AND N. HIRAI, *J. Polym. Sci., Part A-2*, 4 (1966) 161–171.
32 Y. YAMASHITA AND K. MONOBE, *J. Polym. Sci., Part A-2*, 9 (1971) 1471–1481.
33 J.-L. JANE AND J. F. ROBYT, *Carbohydr. Res.*, 132 (1984) 105–118.
34 R. R. BUMB AND B. ZASLOW, *Carbohydr. Res.*, 4 (1967) 98–101.
35 T. D. SIMPSON, F. R. DINTZIS, AND N. W. TAYLOR, *Biopolymers*, 11 (1972) 2591–2600.
36 L. PAULING, *The Nature of the Chemical Bond*, 3rd edn., Cornell University Press, Ithaca, N.Y. 1960.
37 *CRC Handbook of Chemistry and Physics*, 61st edn., CRC Press, Boca Raton, FL, 1980, F-218–221.
38 D. M. SMALL, in D. M. SMALL (Ed.), *Handbook of Lipid Research 4 – The Physical Chemistry of Lipids*, Plenum Press, New York, 1986, pp. 43–49.
39 M. A. WHITTAM, S. G. RING, AND P. D. ORFORD, in G. O. PHILLIPS, D. J. WEDLOCK, AND P. A. WILLIAMS (Eds.), *Gums and Stabilisers for the Food Industry 3*, Elsevier Applied Science Publishers, London, 1986, pp. 555–563.
40 R. STUTE AND G. KONIECZNY-JANDA, *Staerke*, 35 (1983) 340–347.