CALCIUM BINDING BY HYDROXYPROPYL DISTARCH PHOSPHATE AND UNMODIFIED STARCHES[1]

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ABSTRACT

Hydroxypropyl distarch phosphate (HDP) and unmodified starch were held in calcium solutions. The starch was separated centrifugally and the amount of calcium remaining in the supernatant was determined by atomic absorption spectrophotometry. Bound calcium was calculated by subtracting the calcium in the supernatant from the calcium in the original solution. The effects of starch gelatinization, pH, temperature, time, and calcium:starch ratio on binding were studied. Ungelatinized, unmodified starch and HDP bound up to 86 μg calcium/g starch. Binding was influenced markedly by the calcium concentration in the reaction mixture. The pH influenced the degree of binding by HDP, but had no effect on unmodified starch. As the temperature increased from 5° to 45°C, the extent of binding decreased. The amount of calcium bound was constant after a 20-min reaction time. Unmodified tapioca bound more calcium than unmodified corn or waxy maize starch. Gelatinization of both HDP and unmodified tapioca starch nearly eliminated binding. These results suggest that binding of calcium by starch is related to granule structure and that it is principally nonionic in unmodified starch, and both nonionic and ionic in HDP.

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The interaction of metallic cations with carbohydrates has been demonstrated for both ionic and nonionic carbohydrates (1). The complexes formed are referred to as adducts and can occur among alkaline metals (*e.g.*, Li, Na, K), alkaline earth metals (*e.g.*, Be, Mg, Ca), or transition elements (*e.g.*, Fe, Ni, Zn, Cu) and multidonor ligand molecules which contain properly oriented hydroxyl groups.

There has been a limited number of studies on cation binding by starch. Hollo *et al.* (2) suggested that cation binding was related to the phosphate content of starch. Wettstein *et al.* (3) showed that divalent cations were bound by cross-linked starch phosphate and that the selectivity increased in the order Ca < Ni < Zn < Cu.

Metallic cations have been shown to influence the structure of the starch granule. Various cations at relatively high concentrations induce gelatinization or lower the gelatinization temperature of starch (4,5). Leach *et al.* (6) demonstrated that the adsorptive affinity of starch for alkaline metals was not markedly affected by the species of starch, content of the linear fraction, granule size, or micellar organization within the granule.

We have been interested in the ability of starch to complex with metallic cations (7,8). Obviously, such complexing could have nutritional significance and could influence metal-requiring chemical and biochemical reactions in starch-containing foods (9). The results of studies on calcium-starch complexing are reported here.

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MATERIALS AND METHODS

Unmodified and modified (HDP = hydroxypropyl distarch phosphate, MS = 0.045) tapioca starches were obtained from Stein, Hall & Co., Inc., New York, N.Y. Unmodified corn and waxy maize starches were obtained from National Starch and Chemical Corp., New York, N.Y.

In ungelatinized starch experiments, a 2% dispersion of starch was prepared by adding 20 ml $CaCl_2$ or $CaCO_3$ standard solution (1-20 μg Ca^{++}/ml water) to 0.4 g of starch. The dispersion was held at 25°C for 20 min, stirred at 4 min intervals, and centrifuged (7,800 × *g*, 20 min, 5°C). Ten milliliters of supernatant, with 3% La^{+++} (La_2O_3) added, was analyzed for calcium using a Perkin-Elmer Atomic Absorption Spectrophotometer Model 290B or 360. Controls were prepared with 0.4 g starch and 20 ml water.

In gelatinized starch experiments, a 2% starch dispersion was prepared by adding 20 ml $CaCl_2$ standard solution (1-20 μg Ca^{++}/ml water) to 0.4 g starch. The unmodified tapioca starch and HDP were gelatinized at 70°C for 2.5 min and 90°C for 15 min, respectively. The gelatinized starch was held at 25°C for 20 min and centrifuged (43,500 × *g*, 10 min, 5°C). Supernatants were analyzed for calcium according to the standard additions method described in the Perkin-Elmer manual (10). Three per cent La^{+++} (La_2O_3) was added prior to analysis.

The $CaCl_2$ standard solutions used to disperse the starch were pH 6.3. To determine the effect of pH on binding, $CaCl_2$ standard solutions were titrated to pH 3.4 with 0.01 *N* HCl.

The $CaCO_3$ standard solutions were prepared by suspending $CaCO_3$ in water and titrating to pH 3.4 with concentrated HCl. To adjust the pH to 6.3, the standard solutions were neutralized with 0.1 *N* NaOH.

All solutions were prepared from analytical reagent-grade compounds. Distilled-deionized water was used in all experiments.

The amount of calcium bound was calculated as follows:

$$\mu g\ Ca^{++} \text{ in standard solution} - \mu g\ Ca^{++} \text{ in supernatant} = \mu g\ Ca^{++} \text{ bound.}$$

RESULTS AND DISCUSSION

Binding of calcium by ungelatinized, unmodified tapioca starch was influenced markedly by the concentration of calcium ions in the reaction mixture, but did not appear to be affected by pH (Table I). Twenty-five to sixty-four μg of calcium was bound per g of starch, depending on the calcium:starch ratio. At both pH 6.3 and 3.4, the amount of calcium bound increased as the calcium ion concentration increased up to a starting concentration of 250 and 400 μg of calcium/g of starch, respectively. Above 400 μg, the amount bound decreased and the experimental variability increased. We cannot explain the peak at 250–400 μg. When the μg calcium bound was calculated as a percentage of the μg calcium available for binding, the maximum percentage bound was obtained at the lowest calcium concentration (50 μg). As the calcium ion concentration increased from 50 to 1000 μg/g of starch, the percentage bound decreased. Thus, the amount of calcium bound by starch increased up to about 250 to 400 μg calcium, but a decreasing percentage of the available calcium was bound over the same range.

HDP bound more calcium at pH 6.3 than the unmodified tapioca starch, while HDP and unmodified starch bound about the same amount at pH 3.4 (Table II). Therefore, pH appears to influence the amount of calcium bound by HDP. This may be due to the ionic nature of the phosphate groups in the HDP. The amount bound decreased as the pH decreased. As with the unmodified starch, maximum binding was observed at about 250 to 400 μg of calcium/g of starch, and the percentage bound decreased as the calcium ion concentration increased.

The calcium salt employed influenced the amount of calcium bound by either unmodified tapioca starch or HDP (Table III). The magnitude of this effect varied with the pH. At pH 6.3, both starches bound more calcium when $CaCl_2$ was used instead of $CaCO_3$. This cannot be explained on the basis of solubility differences between $CaCl_2$ and $CaCO_3$. The calcium from either source would

TABLE I
Effect of Calcium Concentration and pH on Calcium Binding by Ungelatinized, Unmodified Tapioca Starch

Ca^{++} Concentration[a]	Ca^{++} Bound			
	3.4[b]		6.3	
	μg	%[c]	μg	%
50	25.5 ± 3.5[d]	51.0 ± 6.8	24.0 ± 2.0	48.0 ± 3.8
100	30.0 ± 3.0	30.0 ± 3.0	38.0 ± 1.0	38.0 ± 1.1
150	37.5 ± 5.5	25.0 ± 3.9	47.5 ± 3.5	31.7 ± 2.3
200	54.5 ± 3.5	27.3 ± 1.8	56.0 ± 2.5	28.0 ± 1.5
250	62.5 ± 3.0	25.0 ± 1.3	62.5 ± 3.0	25.0 ± 1.2
400	59.5 ± 6.5	14.9 ± 1.5	64.0 ± 1.5	16.0 ± 0
500	31.5 ± 16.0	6.3 ± 2.9	6.5 ± 12.5	1.3 ± 2.5
750	32.5 ± 15.5	4.3 ± 2.1	18.0 ± 13.0	2.4 ± 1.7
1000	14.0 ± 12.0	1.4 ± 1.1	23.0 ± 13.0	2.3 ± 1.3

[a] μg Ca^{++} added/g starch.
[b] pH of $CaCl_2$ solution.
[c] μg Ca^{++} bound × 100/μg Ca^{++} added.
[d] Mean ± SD, n ≥ 4.

TABLE II
Effect of Calcium Concentration and pH on Calcium Binding by Ungelatinized Hydroxypropyl Distarch Phosphate

Ca^{++} Concentration[a]	Ca^{++} Bound			
	3.4[b]		6.3	
	μg	%[c]	μg	%
50	26.5 ± 1.5[d]	53.0 ± 2.8	34.0 ± 1.0	68.0 ± 1.8
100	33.0 ± 8.5	33.0 ± 8.6	51.5 ± 1.0	51.5 ± 1.0
150	43.0 ± 6.5	28.7 ± 4.4	63.5 ± 2.0	42.3 ± 1.5
200	60.0 ± 2.0	30.0 ± 1.1	68.5 ± 3.5	34.3 ± 1.7
250	62.5 ± 4.0	25.0 ± 1.5	75.0 ± 4.5	30.0 ± 1.8
400	57.0 ± 6.5	14.3 ± 1.4	86.5 ± 7.0	21.6 ± 1.6
500	34.5 ± 4.0	6.9 ± 0.8	63.0 ± 10.0	12.6 ± 1.7
750	15.0 ± 15.0	2.0 ± 2.3	48.5 ± 14.0	6.5 ± 1.9
1000	14.0 ± 16.5	1.4 ± 1.7	42.5 ± 12.0	4.3 ± 1.2

[a] μg Ca^{++} added/g starch.
[b] pH of $CaCl_2$ solution.
[c] μg Ca^{++} bound × 100/μg Ca^{++} added.
[d] Mean ± SD, n ≥ 4.

have been completely soluble in the reaction mixtures at pH 6.3, since the $CaCO_3$ was dissolved in HCl before being adjusted to pH 6.3 with NaOH. The sodium present in the neutralized $CaCO_3$ solution may have competed with the calcium for binding sites in the starch. At pH 3.4, binding of calcium from $CaCl_2$ was still greater than from $CaCO_3$, although the amount bound from either source was less than at pH 6.3.

Incubation temperature influenced the percentage calcium bound in both the ungelatinized HDP and the unmodified tapioca starch (Table IV). As the temperature increased from 5° to 45°C, the amount of binding decreased. The effect was greatest between 5° and 25°C for the unmodified starch, and between 25° and 45°C for HDP. Although the two starches bound different percentages of calcium at 5°C, the same amount was bound at 45°C.

Increasing the reaction time beyond 20 min had no effect on calcium binding by either of the ungelatinized starches (Table V). Maximum binding was attained within 20 min.

Ungelatinized, unmodified starches from different sources bound different amounts of calcium (Table VI). This difference is probably due to differences in granular structure and not to the amylose:amylopectin ratio within the granule, since tapioca and corn starch have about the same amylose content.

Gelatinization of both HDP and unmodified tapioca starch nearly eliminated the ability of the starch to bind calcium (Table VII). This is an example of how the spectrophotometric method applied can drastically affect the analytical results obtained. When the same method was applied to the gelatinized starch as

TABLE III
Effect of Calcium Salt on Calcium Binding by Ungelatinized, Unmodified Tapioca Starch and Hydroxypropyl Distarch Phosphate[a]

		pH[b]	
		6.3	3.4
Unmodified	$CaCl_2$	31.7 ± 2.3[c,d]	25.0 ± 3.9
	$CaCO_3$	25.8 ± 0.5	14.4 ± 1.5
Hydroxypropyl distarch phosphate	$CaCl_2$	42.3 ± 1.5	28.7 ± 4.4
	$CaCO_3$	29.0 ± 0.0	23.0 ± 1.9

[a]150 μg Ca^{++} added/g starch.
[b]pH of $CaCl_2$ and $CaCO_3$ standard solutions was 6.3 and 3.4, respectively. $CaCl_2$ was adjusted to pH 3.4 with HCl, and $CaCO_3$ was adjusted to pH 6.3 with NaOH.
[c]μg Ca^{++} bound × 100/μg Ca^{++} added.
[d]Mean ± SD, n ≥ 4.

TABLE IV
Effect of Incubation Temperature on Calcium Binding by Ungelatinized, Unmodified Tapioca Starch and Hydroxypropyl Distarch Phosphate[a]

Temperature °C	Unmodified	Hydroxypropyl Distarch Phosphate
5	38.3 ± 2.7[b,c]	45.0 ± 6.0
25	31.8 ± 2.3	42.3 ± 1.5
45	30.1 ± 1.7	31.6 ± 2.3

[a]150 μg Ca^{++} added/g starch.
[b]μg Ca^{++} bound × 100/μg Ca^{++} added.
[c]Mean ± SD, n ≥ 10.

was applied to the ungelatinized starch, the results indicated that there was an increase in the amount bound by the unmodified starch and a decrease in the amount bound by HDP. However, when the method of standard additions was used (the method intended to remove errors introduced by viscosity or other interfering factors), the amount of calcium bound by both the gelatinized HDP and the unmodified starches was only 10 to 12%. Thus, the binding capacity of both starches is dramatically reduced by gelatinization. This would support the

TABLE V
Effect of Reaction Time on Calcium Binding by Ungelatinized, Unmodified Tapioca Starch and Hydroxypropyl Distarch Phosphate[a]

Time min	Unmodified	Hydroxypropyl Distarch Phosphate
20	34[b]	43
40	36	43
80	36	40
120	36	41

[a]150 μg Ca^{++} added/g starch.
[b]μg Ca^{++} bound × 100/μg Ca^{++} added.

TABLE VI
Binding of Calcium by Different Ungelatinized, Unmodified Starches[a]

Tapioca	38.1 ± 1.1[b,c]
Corn	27.0 ± 2.0
Waxy maize	15.3 ± 0.6

[a]100 μg Ca^{++} added/g starch.
[b]μg Ca^{++} bound × 100/μg Ca^{++} added.
[c]Mean ± SD, n = 3.

TABLE VII
Effect of Atomic Absorption Spectrophotometric Method on Apparent Amount of Calcium Bound by Unmodified Tapioca Starch and Hydroxypropyl Distarch Phosphate

	Method			
	Direct		Standard Additions	
	150 μg[a]	500 μg	150 μg	500 μg
Ungelatinized				
Unmodified	31[b]	...	32	...
Hydroxypropyl distarch phosphate	43	...	42	...
Gelatinized				
Unmodified	61	60	12	7
Hydroxypropyl distarch phosphate	28	29	10	0

[a]μg Ca^{++} added/g starch.
[b]μg Ca^{++} bound × 100/μg Ca^{++} added.

hypothesis that binding of calcium by starch is related to the granular structure. It would appear from these results, as well as from electron microscopy observations on iron-starch complexing (8), that cations are adsorbed to the surface of the ungelatinized tapioca starch granule and do not significantly penetrate into the granule. Gelatinization apparently alters the granule surface structure to the degree that binding is drastically reduced.

The fact that HDP binds greater amounts of calcium than unmodified tapioca starch and that pH has an effect on HDP but not on unmodified starch suggests that the level of binding by HDP above what was observed in the unmodified starch may be ionic binding. Thus, cation binding by unmodified starch may be essentially nonionic, whereas it is both nonionic and ionic in HDP.

Although our results (7,8) suggest that very low levels of minerals are bound by starch, binding may be significant nutritionally in light of the observed differences in *in vitro* digestibility between HDP and unmodified starch (11). Minerals bound by starch in a food product may be biologically unavailable if the starch is not completely digestible. We are carrying out animal studies to determine whether or not starch (in either the ungelatinized or the gelatinized form) can influence mineral bioavailability.

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