CCXIV. COMPOUNDS OF SERUM PROTEINS WITH POLYSACCHARIDES.

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THE proteins contain, apart from the CONH group, a large number of the most varied side-chains derived from amino-acid residues. According to Przyłęcki [1932] the side-chains form in proteins, together with the binding group CONH, fourteen typical groups. Thanks to the presence of these groups, the proteins possess affinities with a large number of the most varied substances, such as (1) compounds possessing polar groups (polysaccharides, fats, lecithins), (2) compounds possessing non-polar groups, such as: light petroleum or other hydrocarbons, benzene, toluene, cholesterol, etc. The presence or absence of certain groups, as also their heterogeneous mutual position contribute to cause the greatly differing behaviour of the various proteins. The investigations of Hardy [1905] and Pauli & Valkó [1933] furnish classical examples of the functional relation between the structure and the properties of proteins. Przyłęcki and his co-workers in a long series of papers [1931-34] have examined the relation between the composition of proteins and their ability to form compounds with lipoids, fats, nucleic acids and polysaccharides; special regard was paid to the dependence of binding on the presence and amount of amino-acids, and their distribution in the protein molecule. According to Przyłęcki [1932] it is necessary to distinguish two types of binding between proteins and other substances, viz.:

A. Complex compounds formed by the binding of polar groups of proteins with the polar groups of other substances free of ionized groups (Willstätter's *symplexes*).

B. Compounds of the multi-polar type, formed by the binding of ionized groups (e.g. H_3PO_4 , amylopectin etc.) with the ionized groups contained in the protein similar to the salts.

In order to solve the problem of binding between proteins and various non-ionized groups, Przyłęcki *et al.* [1935; 1936] conducted two types of experiments.

I. Polysaccharides (phosphoric acid-free) were bound with pure amino-acids and with synthetic polypeptides. The formation of compounds between amylose and various amino-acids was examined by means of polarimetric, refractometric, cataphoretic, cryoscopic and ultrafiltration methods. The results attained indicated that the following amino-acids do not combine with amylose:

- (a) glycine, alanine, valine, leucine;
- (b) aspartic acid, glutamic acid, asparagine;
- (c) cystine, phenylalanine, tryptophan, hydroxyproline, lysine and histidine;
- (d) the CONH group (pentaglycylglycine, leucylglycylglycine, glycylglycine, glycylalanhydride).

Amongst the amino-acids, the following are exceptions: tyrosine and arginine (with its guanidine group). Tyrosine, for instance, could bind as much as 20-40 % of the polysaccharide.

On the basis of these experiments, it was shown that of the large number of side-chains and the binding group (CONH) entering into the composition of the protein molecule, only arginine and tyrosine combine with polysaccharides. Experiments on free amino-acids cannot of course yield proof that only the above amino-acids can be taken into account in the binding of polysaccharides with proteins, since:

(1) Free amino-acids do not necessarily react in the same way as when forming part of a protein molecule.

(2) Not all the amino-acids entering into the composition of the protein molecule have been identified.

(3) Many proteins contain various prosthetic groups or contaminants.

For these reasons a second series of experiments was undertaken, a part of which is described herein, the rest having been given by Przyłęcki *et al.* [1936].

II. In most of the experiments the results with free amino-acids or with polypeptides were in agreement with those for the corresponding proteins. These experiments indicate that some proteins bind much amylose, whilst others bind or adsorb only insignificant amounts. The positive role of arginine and tyrosine was also proved, as only those types of protein bound amylose which contained large quantities of tyrosine (caseinogen 5.4 %, serum globulin 6.7 %, silk fibroin 11.0 %), or of arginine (clupein with 87 % arginine yielded as much as 70–90 % of amylose in the precipitate).

Of the serum proteins examined by Przyłęcki *et al.* [1931–36] globulin bound amylose most strongly, while albumins bound it only under certain given conditions. As the experiments on the combination of serum protein with amylose were conducted under various conditions and with various methods, using substances containing lipoids, it was sought to elucidate the following points:

(1) how far the various kinds of experimental methods used affect the binding;

(2) which of the globulins, whether eu- or pseudo-, has the greatest binding capacity;

(3) whether phosphorus-free amylose is also bound by serum proteins;

(4) whether the fat content of the serum proteins affects their ability to bind amylose.

EXPERIMENTAL.

Horse and ox serum proteins.

Separation was effected by fractional precipitation with $(\text{NH}_4)_2\text{SO}_4$, and purification by thrice-repeated solution and re-precipitation. The protein was then dialysed for 3 days against ordinary water and for 2 days against distilled water and then dried *in vacuo* over sulphuric acid at 35°.

Phosphorus-free amylose was prepared by electro dialysis for 12 hours of 2.5 % solution of rice starch in a Pauli electro dialyser at about 400 v. and 30–40 milliamps.; the solution was then evaporated at pH 7, to the desired concentration over an ordinary Bunsen burner. Phosphorus was in all cases found to be absent.

Fat-free protein was prepared by Young's method and in addition protein was used from which the fat was extracted by means of ether and methylene chloride. The fat contents of the proteins were determined by the method of Kumagayo-Suto.

Phosphoric acid in the amylose was determined by the help of ammonium molybdate after prior evaporation to dryness of the amylose with the addition of HNO_3 .

2% solutions or suspensions of proteins were used in the experiments, of which there were two series.

In the first series the protein was mixed with a 0.22% solution of amylose, and after standing for 15 min. was precipitated by boiling or by changing the pH and then filtered out.

In the second series the protein was coagulated either by heating or by changing the pH before adding the amylose; 0.2% (approx.) solution of amylose was added to a 2% suspension of protein, brought to a suitable pH and the whole filtered after 15 min.

The combination between the protein and the amylose was determined by changing the concentration of amylose in the filtrate. The amylose in the filtrate was determined by the method of Pflüger. The tables give the percentage diminution in amylose concentration and the quantity of amylose bound by the protein in percentages of the latter.

In addition, experiments were conducted in the presence of neutral salts (CaCl_2 , Na_2SO_4 and KCl).

RESULTS.

Protein coagulation and the binding of amylose by serum albumin.

In order to ascertain whether protein coagulated by boiling or by change of pH possesses the same ability to bind amylose as protein in the form of a sol, the following series of experiments was made:

(1) 50 ml. 2% suspension of ox albumin + 50 ml. of 0.22% solution of amylose + 0.5 g. of Na_2SO_4 (pH 4.0).

(2) 50 ml. 2% suspension of ox albumin + 50 ml. of amylose + 0.5 g. CaCl_2 (pH 7.7).

(3) Same as No. 2, but pH 8.0.

(4) 50 ml. of ox albumin solution + 50 ml. amylose + 0.5 g. CaCl_2 (pH 7.7). Albumin precipitated by heating.

(5) Same as No. 4, but pH 8.0.

(6) 50 ml. of ox albumin solution + 50 ml. of amylose + 1% Na_2SO_4 (pH 4.0).

(7) Same as No. 6, but albumin precipitated by heating, pH 8.0.

(8) 50 ml. of ox albumin solution + 50 ml. amylose (pH 6.17). The albumin solution before the addition of amylose was denatured without precipitation by heating after adding 5 ml. of $N/10$ NaOH and then precipitated by change of pH.

(9) Same as No. 8, but with horse albumin, pH 5.52.

It follows from the data in Table I that once albumin is coagulated it cannot bind amylose. If, however, the protein is in the form of a sol, a slight combination ensues. The addition of CaCl_2 does not specially affect the binding

Table I.

Exp. no.	pH	Salt added	Diminution in amylose concentration %	Amylose bound by protein (computed in % of protein)
1	4.0	0.5% Na_2SO_4	0	6.0
2	7.7	0.5% CaCl_2	0	0
3	8.0	0.5% CaCl_2	0	0
4	7.7	0.5% CaCl_2	21.5	2.25
5	7.7	0.5% CaCl_2	25.5	2.2
6	4.0	0.5% Na_2SO_4	0	0
7	8.0	2% Na_2SO_4	29.2	3.4
8	6.17	0	28.9	2.6
9	5.52	0	35.1	4.8

between the albumin and the amylose as when using Na_2SO_4 we have very closely similar results. It is further seen that binding occurs only at moderately low $p\text{H}$; thus at $p\text{H}$ 4 there is no binding, whereas at $p\text{H}$ 5.52 binding occurs. If we denature the protein (without precipitating it by heating at a high $p\text{H}$), mix with amylose and then precipitate by change of $p\text{H}$, the figures obtained are practically the same as those for albumin solution precipitated by boiling (cf. Exp. 8 with Exps. 4 and 5). Horse albumin binds more strongly than ox albumin.

Fat content and the binding of amylose with pseudoglobulins.

In former researches, it was only found that globulins (both eu- and pseudo-) bind amylose strongly. In the present series of experiments it was sought to determine to which of the globulins this property should be ascribed, and whether the fat content affects binding. With these objects in view we took ordinary globulin and globulins defatted by the method of Young and by that of extraction with ether and methylene chloride. The following experiments were then carried out:

(1) 2% suspensions of pseudoglobulin (horse) prepared by boiling in the presence of 2% Na_2SO_4 ; 25 ml. suspension + 25 ml. amylose ($p\text{H}$ 4.0).

(2) Same as No. 1, but $p\text{H}$ 7.0.

(3) 2% solution of pseudoglobulin (horse) dissolved at $p\text{H}$ 8.5, amylose added and globulin precipitated at $p\text{H}$ 5.0.

(4) 2% solution of pseudoglobulin (defatted by extraction with ether and methylene chloride), dissolved in 4% KCl , amylose added, and precipitated by boiling ($p\text{H}$ 4.0).

(5) Same as No. 4, but $p\text{H}$ 8.0.

(6) Pseudoglobulin (horse), defatted by Young's method, dissolved at $p\text{H}$ 8.5, amylose added, globulin precipitated at $p\text{H}$ 6.2; after filtering, the extent of combination was examined by determining the loss in concentration of amylose.

Table II.

Exp. no.	$p\text{H}$	Concentration of amylose %	Change in con- centration of amylose %	Amylose bound by 100 g. of protein g.	Diminution between control and experiment mg.
1	4	0.11	7.7	0.8	0.5
2	7	0.11	8.1	0.9	0.5
3	5	0.1	13.1	1.2	2.4
4	4	0.11	3.7	0.45	0.9
5	8	0.11	3.1	0.4	0.8
6	6.2	0.15	0	0	0

It can be seen from this table, excluding Exp. 3, that pseudoglobulin and phosphoric acid-free amylose do not combine with each other. The values obtained are so small that they must be regarded as lying within the limits of experimental error. On the basis of these figures we can also conclude that the lipid content of pseudoglobulin has only a small influence on the binding of amylose.

Binding of euglobulin with amylose.

The following experiments were made with euglobulin (ox):

(1) A suspension of undefatted euglobulin was prepared by dissolving at a high $p\text{H}$, then precipitated by the addition of HCl , and an amylose solution was added to the suspension. 25 ml. of 2% suspension of euglobulin + 25 ml. amylose solution ($p\text{H}$ 6.2)

(2) A solution of ox euglobulin, prepared as above, was taken; amylose was added and the protein then precipitated by the addition of HCl; 25 ml. of 2% solution of euglobulin + 25 ml. amylose solution (*pH* 6.2).

(3) An undefatted euglobulin solution was prepared as above; $N/3$ KCl was added and then amylose solution, after which the protein was precipitated by change of *pH* (*pH* 3.6).

(4) A solution of euglobulin, defatted by extraction with ether and methylene chloride and otherwise prepared as above, was taken, amylose was added and precipitation was effected at *pH* 5.0.

(5) A solution of ox euglobulin (defatted by Young's method) and prepared as above was taken; amylose was added and precipitation effected by change of *pH* (*pH* 6.2).

(6) Same as No. 5, but horse euglobulin used and *pH* 4.0.

It can be seen from Table III that it is the euglobulin constituent of globulin that causes strong binding with amylose, noticed in previous researches. Here, as

Table III.

Exp. no.	<i>pH</i>	Initial concentration of amylose	Diminution in final concentration of amylose	Amylose bound with protein %
1	6.2	0.16	9.5	1.5
2	6.2	0.15	48.2	7.7
3	3.6	0.13	68.8	8.95
4	5.0	0.13	0	0
5	6.2	0.17	42.1	7.65
6	4.0	0.1	68.6	7.35

with albumins, it is necessary to state that once the globulins are coagulated, they bind with amylose more weakly. It is also shown that fat has no significance in binding amylose. The fact that euglobulins extracted with ether and methylene chloride fail to bind with amylose requires further investigation.

DISCUSSION.

Earlier research on the binding of proteins with polysaccharides showed that not all of the former bind the latter. The present research has made it possible to extend this observation, viz. it has been shown that under identical conditions of binding the serum albumins bind amylose deprived of H_3PO_4 only in a very small degree, whilst of the globulins, only euglobulin binds, pseudo-globulin not doing so to any appreciable extent.

It can therefore be stated that the chemical composition of these two globulins is fundamentally different as the binding between polysaccharides does not depend (as was previously shown) on the colloidal properties of proteins (size and shape of molecules), but on their composition, i.e. on the composition of amino-acids, their distribution in the molecule and their ability to enter into the reaction.

It can also be confirmed that the bindings between proteins and amylose are not always of a heteropolar nature. De-lipoided proteins bind just as do those containing fat (cholesterol, lecithin, aliphatic acids). It hence follows that amylose is not bound by the lipid groups attached to the protein.

It is of interest that coagulation of serum protein before the addition of amylose, even in the case of euglobulin with its strongly binding action, results in the binding being so weak that no certain conclusion as to its existence can be

drawn. This is perhaps due to a mutual interaction of those groups which are responsible for combination with the amylose, or possibly also to their molecular grouping being re-arranged in such fashion that combination becomes impossible.

SUMMARY.

1. Phosphoric acid-free amylose is able to bind with proteins.
2. Euglobulin is the only serum globulin which binds with polysaccharides free of ionized groups; pseudoglobulin practically does not bind, whilst the albumins combine only in a very small degree.
3. Albumin coagulated by boiling or by change of *pH* does not bind amylose.
4. Fats do not affect combination between serum euglobulins and amylose, as those containing fat bind just as well as those deprived of it by Young's method.

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