# Lupin seed proteins

## III. A PHYSICO-CHEMICAL STUDY OF THE PROTEINS FROM WHITE LUPIN SEED (*LUPINUS ALBUS*)

In parts I[1] and II[2] physico-chemical studies of the proteins from blue and yellow lupin seed were respectively reported. The present communication describes some preliminary results obtained with the proteins of white lupin seed (*Lupinus albus*).

Decorticated white lupin seed meal was defatted and purified as described[1]. The proteins of the purified lupin seed meal were completely extracted with 10% sodium chloride and then precipitated by adding ammonium sulphate to 85% saturation. Measurements on a Spinco electrically driven ultracentrifuge, revealed (Fig. 1(a)) one major component of sedimentation constant of 8.7 Svedberg Units (S.U.) and two minor components of 12.2 and 2.7 S.U. (respectively designated as the $s_{8.7}$, $s_{12.2}$ and $s_{2.7}$ components), for this preparation in phosphate buffer of ionic strength ($I$) = 0.31, pH = 7.0.

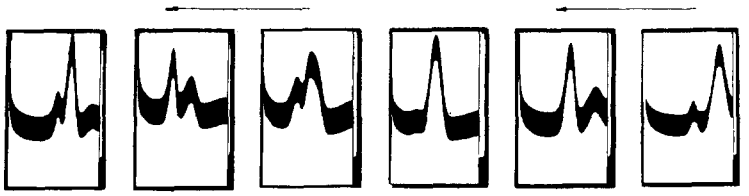


Fig. 1. Sedimentation diagrams of white lupin seed protein fractions in phosphate buffer $I$ = 0.31, pH = 7.0.

The globulins of the purified white lupin seed meal showed little response to the fractionation procedure described[1] for blue lupin seed globulins. A more successful fractionation was obtained by adding successive amounts of saturated ammonium sulphate to a 10% sodium chloride purified lupin seed extract. Fractions A, B, C, D and E were respectively obtained at 31%, 40%, 50%, 70% and 85% saturation with ammonium sulphate and were examined in the ultracentrifuge in buffer $I$ = 0.31, pH = 7.0. Fraction A (5% of total globulins) contained a major $s_{12.2}$ component with a smaller amount of the $s_{8.7}$ component (Fig. 1(b)). Fraction B (65% of total globulins) consisted of a major $s_{8.7}$ component and a smaller amount of the $s_{12.2}$ component (Fig. 1(c)). The main peak of fraction B was rather asymmetric and showed a slower trailing edge. For fraction C (15%), a single $s_{8.7}$ component was found (Fig. 1(d)). Fig. 1(e) shows the sedimentation diagrams of fraction D (5%), which consisted mainly of the $s_{8.7}$ component with a smaller amount of the $s_{2.7}$ component. The E fraction (10%) (Fig. 1(f)), on the other hand, contained mainly the $s_{2.7}$ component. This fractionation shows that the $s_{8.7}$ component is by far the dominant component of the globulins. It is only possible, however, to isolate a relatively small part of this component in a reasonably pure form by ammonium sulphate fractionation. The $s_{2.7}$ component is fairly pure in fraction E, but the $s_{12.2}$ component in fraction A does not represent a satisfactory separation.

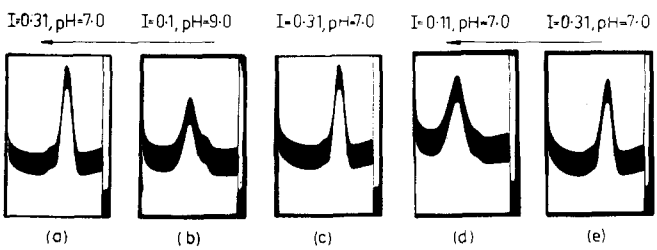


Fig. 2. Sedimentation diagrams of the fraction C in different buffers.

A more detailed examination was carried out on fraction C. Fig. 2(a) showing a single $s_{8.7}$ component gives the sedimentation diagram of fraction C in phosphate buffer $I$ = 0.31, pH = 7.0.

This solution was dialysed overnight against borate buffer $I = 0.1$, pH = 9.0 (Fig. 2(b)). The $s_{8.7}$ component is hereby quantitatively converted into a component of sedimentation constant of 12.2 S.U. Next, this solution was dialysed back to $I = 0.31$, pH = 7.0 and, as shown in Fig. 2(c), the $s_{8.7}$ component formed again and the obtained sedimentation diagrams were exactly similar to the original ones (compare Fig. 2(a) and 2(c)). Hence, the $s_{8.7}$ component forms a reversible association-dissociation system. In the described association reaction of the $s_{8.7}$ component both the ionic strength and pH were altered; the pH was increased from 7.0 to 9.0 and the ionic strength decreased from 0.31 to 0.1. Consequently fraction C was examined in phosphate buffer $I = 0.11$, pH = 7.0, *i.e.*, at the same pH but lowered ionic strength, the sedimentation diagrams obtained (Fig. 2(d)) show a single component of sedimentation constant of 12.8 S.U. When this solution was dialysed back to $I = 0.31$, pH = 7.0, a single $s_{8.7}$ component formed again (Fig. 2(e)). It would thus appear that the decrease in salt concentration caused the association of the $s_{8.7}$ component.

In Table I sedimentation and electrophoretic constants of the globulins from the three lupins studied have been summarized. In the case of the blue and yellow lupins there are close analogies. Each contains a high molecular weight globulin (first globulin) as main constituent, which can be reversibly dissociated; and a "second globulin" which, though similar in molecular weight to dissociated parts of the "first globulin", is distinct and is devoid of association-dissociation properties. Yellow lupin has also a low molecular weight "third globulin". The main constituent of white lupin is an association-dissociation globulin which is not the same as the "first globulin", since the latter associates when the ionic strength is *increased*. It seems rather to replace the "second globulin". It is of interest to note that its electrophoretic mobility resembles that of the "second globulin", even though this is measured under conditions where it is associated.

TABLE I

COMPARISON OF THE SEDIMENTATION AND ELECTROPHORETIC PROPERTIES OF THE GLOBULINS FROM THE THREE DIFFERENT LUPINS

| *Lupin* | *Component* | *Sedimentation constants ($s^0_{20}$-values) in different buffers* | | *Electrophoretic mobility $\times 10^{-4}$ cm$^2$ sec$^{-1}$ volt$^{-1}$ in buffer $I = 0.1$, pH = 9.0* | |
|---|---|---|---|---|---|
| | | $I = 0.31$, pH = 7.0 | $I = 0.1$, pH = 9.0 | *Descending* | *Ascending* |
| *Lupinus angustifolius* (Blue) | 1st Globulin | 11.6 | 7.2* | 1.16 | 1.33 |
| | 2nd Globulin | 7.8 | 7.8 | 0.67 | 0.76 |
| *Lupinus luteus* (Yellow) | 1st Globulin | 11.6 | 7.2* | 1.14 | 1.25 |
| | 2nd Globulin | 7.4 | 7.9 | 0.76 | 0.86 |
| | 3rd Globulin | 2.0 | — | 1.39 | 1.57 |
| *Lupinus albus* (White) | 1st Globulin (?) | 12.2(?) | — | — | — |
| | 2nd Globulin (?) | 8.7 | 12.5* | 0.66 | 0.70 |
| | 3rd Globulin | 2.8 | 3.2 | 1.47 | 1.70 |

* These represent reversible dissociation-association systems.

The small fraction A was not pure, but showed association with increase of ionic strength. It could not be characterized further, but its main constituent is presumably analogous to the "first globulin" of other lupins, present in this case as a secondary constituent of the total globulins. The low molecular weight $s_{2.7}$ component of white lupin is probably analogous to the third globulin of yellow lupin.

White lupin thus appears to contain two dissociating globulins of similar molecular weight, but which are affected in opposite ways by variations of ionic strength. Work at present in hand is directed to a more complete separation and characterization of these two globulins.

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[1] F. J. JOUBERT, *Biochim. Biophys. Acta*, 16 (1955) 370.
[2] F. J. JOUBERT, *Biochim. Biophys. Acta*, 17 (1955) 444.

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