AGGREGATION AND FRAGMENTATION OF SOYBEAN AGGLUTININ

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SUMMARY

Upon storage in the lyophilized state, soybean agglutinin forms high molecular weight aggregates. The aggregation results in a marked increase in hemagglutinating activity, particularly when tested with untrypsinized rabbit or human erythrocytes. Highly purified preparations of soybean agglutinin contain low molecular weight polypeptide fragments, resulting probably from proteolytic cleavage of the intact subunit.

INTRODUCTION

For some time, we have observed that different preparations of highly purified soybean agglutinin[1] differ in their specific hemagglutinating activity, especially when tested with untrypsinized rabbit or human erythrocytes. A systematic study of this phenomenon has shown that upon storage in the lyophilized state SBA forms molecular aggregates which exhibit higher hemagglutinating activity than the native protein, as we had previously found for polymers of SBA obtained by crosslinking with glutaraldehyde (1). In the course of this study we have also observed that preparations of SBA contain low molecular weight polypeptide fragments, which probably result from cleavage of the intact SBA subunit (2).

METHODS

SBA was prepared by affinity chromatography as described before (3) and was kept in lyophilized state at room temperature. Protein concentration was calculated from the absorbance at 280 nm using $A^{1\%}_{280} = 12.8$ (2) or determined according to Lowry et al. (4).

Procedures for the determination of hemagglutinating activity (5,6), for gel filtration on Sephadex G-150 superfine (2) and for polyacrylamide gel electrophoresis (1,2) were those routinely used in our laboratory. The rate of hemagglutination was measured at 24° using a Fragiligraph, model D-2 (Elmedix Ltd., Tel Aviv, Israel), equipped with a linearization unit, according to the method of Danon et al. (7).

[1] Abbreviations used: SBA - soybean agglutinin; HU - hemagglutinating units.

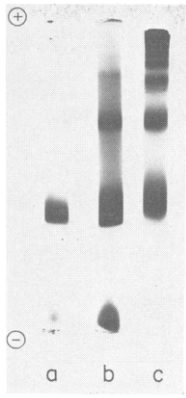

Figure 1: Polyacrylamide gel electrophoresis of SBA. Electrophoresis was on 7.5% gels (0.5 x 8 cm) using an acidic buffer system, pH 4.3 (8). The gels were run at 2 mA per gel for 5 h, and then stained for protein with Coomassie Brilliant Blue R-250. Gel a - 100 µg of freshly prepared SBA; b and c - 200 µg of preparations stored for 8 and 14 months, respectively. Direction of migration was from top. The lowest bands in gels a and b are the tracking dye, methyl green.

RESULTS AND DISCUSSION

Polyacrylamide gel electrophoresis of freshly prepared SBA (preparation a) and of two preparations (b and c) which had been stored for 8 and 14 months respectively, is shown in Fig.1. As can be seen, freshly prepared SBA (a) migrates as a single band, whereas in the stored preparations additional bands of higher molecular weight appear (b,c). Preparations b and c were used by us in our previous studies on the physicochemical properties of SBA (2); immediately after their isolation they migrated on gel electrophoresis as single bands, exactly as preparation a in Fig.1. Thus it is clear that the number of bands and their molecular weight increase with the time of storage.

Marked differences were also observed in the hemagglutinating activity of the three preparations when measured with untrypsinized rabbit erythrocytes. The freshly prepared SBA (a) had a specific activity of 24 hemagglutinating units[1] per mg (for the definition of HU see refs. 5,6) which is in the range found earlier for fresh preparations of SBA (20 to 40 HU/mg); preparations b and c had specific activities of 100 and 180 HU/mg, respectively.

When preparation c was subjected to gel filtration on a column of

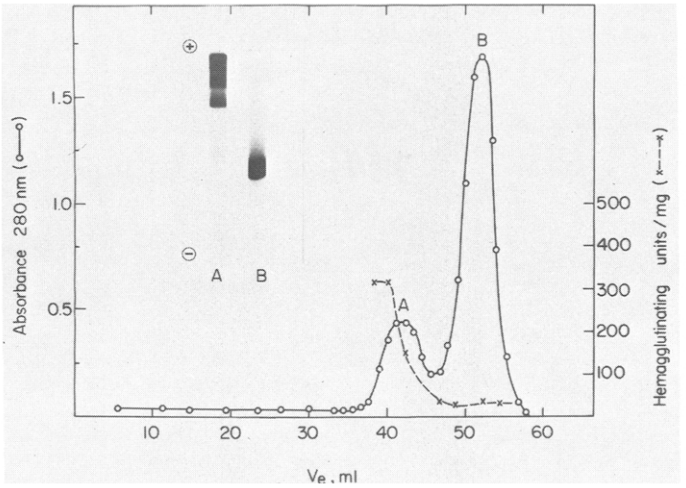

Figure 2: Separation of aggregated SBA from the native lectin by gel filtration. Preparation c (15 mg in 1 ml of 0.9% NaCl) was applied to a column (1.9 x 45 cm) of Sephadex G-150 superfine and eluted with 0.9% NaCl; fractions of 1 ml were collected at a flow rate of 3 ml per hour. The absorbance at 280 nm of all fractions and the hemagglutinating activity of selected fractions, were measured. Inset: Gel electrophoresis of 100 µg protein from pooled peaks A and B carried out as described in Fig.1.

Sephadex G-150 superfine, two protein peaks emerged (Fig.2). The specific hemagglutinating activity of the retarded peak (Fig.2,B) tested with untrypsinized rabbit erythrocytes was constant throughout (30 HU/mg). Gel electrophoresis of the material obtained from peak B (fractions eluted between 47 and 55 ml) after dialysis and lyophilization showed a single band (Fig.2, inset B) migrating at the same rate as freshly prepared SBA (Fig.1,a). The specific hemagglutinating activity of the fractions emerging in peak A was higher than that of peak B, and increased with the molecular size of the protein eluted. The highest activity (320 HU/mg) was found in the first protein fractions. Gel electrophoresis of this peak (fractions eluted between 39 and 44 ml), showed that it contained only high molecular weight aggregates of SBA (Fig.2, inset A).

Rechromatography of the materials obtained from peaks A and B after dialysis and lyophilization, gave single symmetrical peaks with the same elution volumes as found with the original mixture, indicating that no

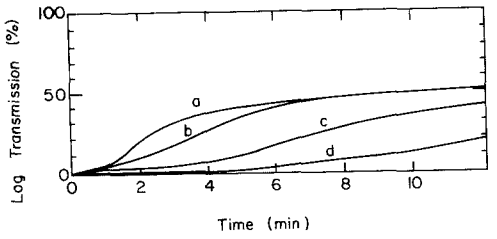

Figure 3: Rate of agglutination of freshly drawn human type A erythrocytes by SBA, measured in the Fragiligraph. To a suspension (2 ml) of erythrocytes (packed cells diluted 1:500 in phosphate buffered saline pH 7.2) was added 0.4 ml of a solution containing: aggregated SBA (peak A), a - 1,200 µg; c - 150 µg; or native SBA (peak B), b - 1,200 µg; d - 150 µg. The increase in light transmission resulting from agglutination was recorded automatically (7).

association or dissociation occured during lyophilization or gel filtration.

The specific hemagglutinating activity of the aggregated forms of SBA (peak A) tested with trypsinized rabbit erythrocytes was 17,000 - 20,000 HU/mg, which is about two times higher than that of native SBA (7,000 - 8,000 HU/mg).

With the aggregated SBA (Fig.3, a,c) both at high (200 µg/ml) and low (25 µg/ml) concentrations, hemagglutination of untrypsinized, freshly drawn, human type A erythrocytes, was much faster than with the native lectin (Fig.3, b,d).

The aggregated forms of SBA gave a maximal mitogenic stimulation of neuraminidase treated mouse spleen lymphocytes (9) at concentrations two to four times lower than the native lectin.

Since SBA is devoid of SH groups and S-S bonds (2), the aggregation observed must be the result of an interaction of other groups. Treatment of aggregated SBA with 0.1% sodium dodecyl sulfate for 3 min at 100°, conditions leading to almost complete dissociation of native SBA into its subunits (2), resulted in incomplete dissociation of the aggregated form (Fig.4, I and II). This finding suggests that the bonds holding the aggregates together are stronger than those responsible for the tetrameric structure of native SBA.

In the latter experiments, the amounts of SBA loaded on the gels were

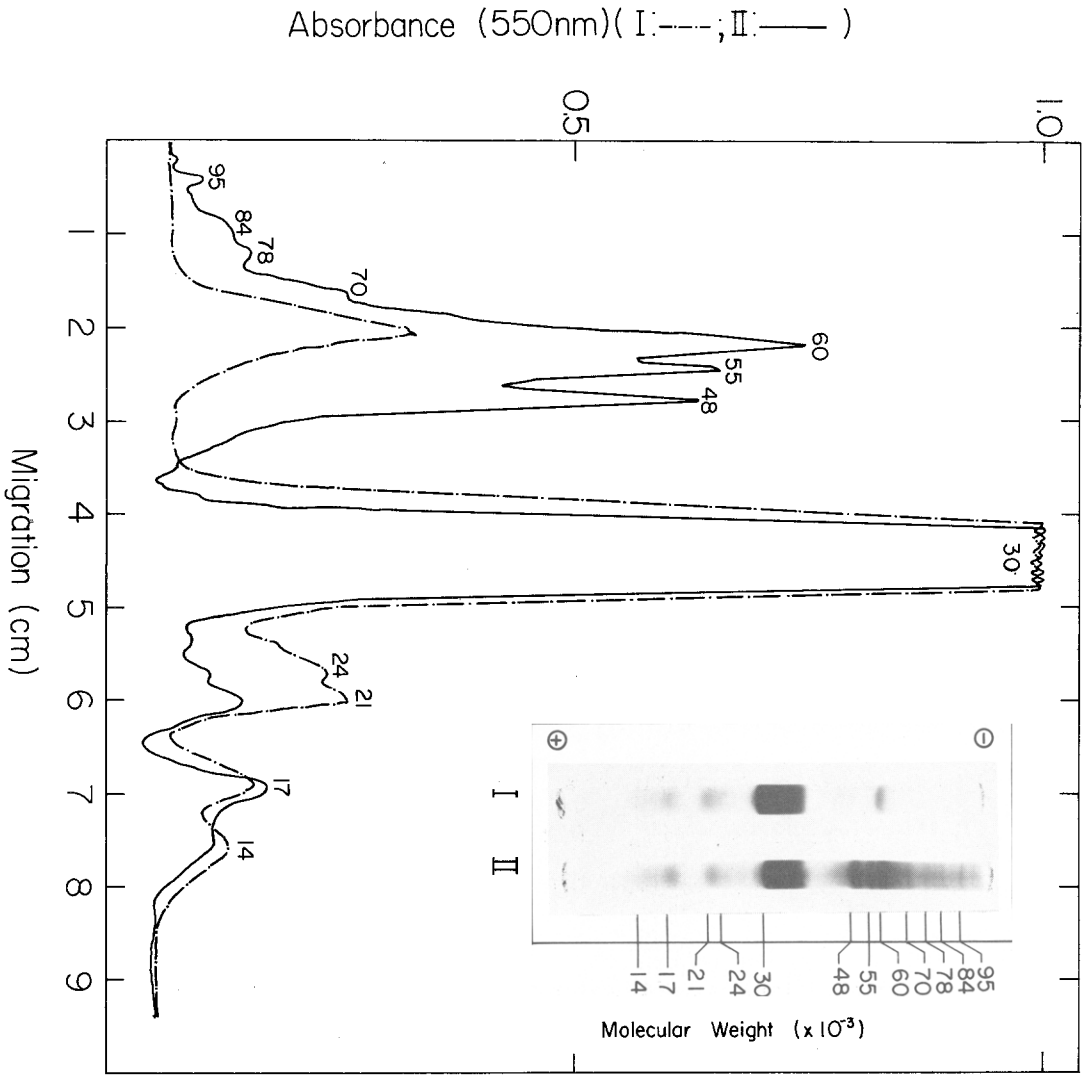

Figure 4: Gel electrophoresis of SBA in the presence of 0.1% sodium dodecyl sulfate at pH 7.2, according to Weber and Osborn (10). I - native SBA (200 µg of protein from peak A, Fig.2); II - aggregated SBA (200 µg of protein from peak B, Fig.2). After electrophoresis the gels were fixed and stained in 12.5% CCl_3COOH. Molecular weights were calculated from the migration of standard proteins under the same conditions. Migration was from top. Densitometric tracing was performed with a Gilford model 2400-S scanner at 550 nm.

much higher than those used in our previous studies on the subunit structure of SBA (2) and the gels were fixed and stained in 12.5% CCl_3COOH. This technique proved to be more sensitive than the one used before, and as a

result four new, fast moving bands were observed. These bands migrated at rates corresponding to molecular weights of 14-24,000.

The low molecular weight fragments were separated from the intact subunit by gel filtration on a column of Sephadex G-150 superfine equilibrated with 0.1% sodium dodecyl sulfate (Fig.5). The amino acid composition of the low molecular weight material (fractions eluted between 110 and 120 ml, Fig.5) thus obtained was very similar to that of native SBA (2), strongly suggesting that these are fragments of the 30,000 molecular weight subunit, rather than contaminants. These fragments may be analogous to those found in concanavalin A (11). However, in SBA the fragments constitute less than 10% (W/W) of the total protein, while in concanavalin A they comprise 40% of the weight of the protein. It is also possible that the protein bands with molecular weights of 48,000 and 55,000, as well as those with molecular weights between 60,000 and 95,000 observed upon gel electrophoresis in sodium dodecyl sulfate (Fig. 4, II), represent aggregates of the native subunit with one of the above fragments.

In view of the marked differences in the biological activities of native SBA and the aggregates that are formed upon storage of the lectin, it is

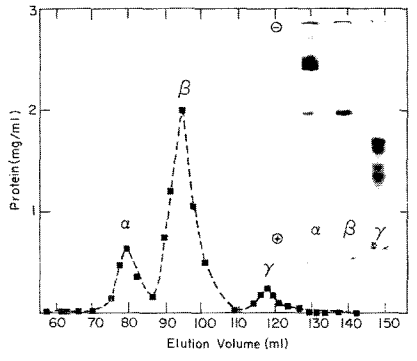

Figure 5: Gel filtration of SBA in the presence of sodium dodecyl sulfate. SBA (25 mg of a fresh preparation) in 1 ml of 0.9% NaCl containing 0.1% sodium dodecyl sulfate was applied to a column (1.85 x 100 cm) of Sephadex G-150 superfine equilibrated in 0.9% NaCl containing 0.1% sodium dodecyl sulfate. Elution was with the same solution; fractions of 2.5 ml were collected at a flow rate of 3 ml per hour. Protein was determined by the method of Lowry et al. (4). Inset: Gel electrophoresis of samples (50 µg protein) from each of the tubes marked (α,β,γ) under the conditions described in the legend to Fig.4.

essential to examine each sample of SBA for homogeneity on polyacrylamide gel electrophoresis before use. Such procedures should also be applied to other lectins which may undergo aggregation upon storage in the dry form or in solution [e.g. concanavalin A (12)]. Aggregation phenomena of this type may be responsible in part for the variations in the biological activities of the same lectins reported by different laboratories (13,14).

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