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An artefact caused by the binding of protein to dextran gel

Some proteins are strongly bound to dextran gel (Sephadex) in environments of low ionic strength[1]. We have noted that this can lead to a serious error when gel filtration is used to assess the dispersity of a protein solute[2]. The error arises from the fact that aggregates of one protein but of different sizes are in general bound in different proportions. Some examples illustrate this and indicate a remedy.

Our current experiments are concerned with immunoglobulins and their peptide chains. Buffers of low ionic strength are used in order to keep the heavy chains monodisperse, and an assessment of dispersity is often carried out by filtration through Sephadex G-150. One such examination of human immunoglobulin G (Cohn Fraction II, Commonwealth Serum Laboratories, Melbourne, Australia) on a freshly packed column is illustrated by curve A in Fig. 1. The protein was eluted as a single symmetrical peak,

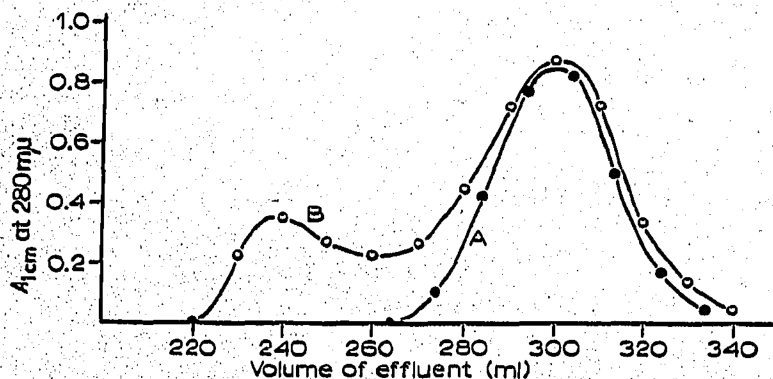

Fig. 1. Elution profiles given by human immunoglobulin G when two aliquots of one solution were passed successively through a column of Sephadex G-150, 3 × 90 cm, equilibrated with 4 m*M* sodium acetate buffer, pH 5.4. Curve A: first passage through a freshly packed column. Curve B: second passage, the column having been washed with 2 l of the acetate buffer between the runs. In each case 40 mg of protein was applied in 5 ml of the buffer.

suggesting that it was completely monodisperse. Such a finding, however, was at variance with ultracentrifugal analyses and examination of a second aliquot on the column (curve B, Fig. 1), both of which indicated the presence of about 20% aggregate. It is important to note that no change of buffer had intervened between the two runs in Fig. 1. The recovery of protein from the first run was 64%, from the second $> 95\%$. Third and fourth runs, again with no intervening change of buffer, gave the same results as the second run.

This disparity between the first and subsequent runs through the column could be reproduced precisely after the column had been washed with 0.1 *M* NaOH, thereby eluting all the bound protein. The capacity for binding immunoglobulin G in the presence of the acetate buffer was approximately 0.03 mg protein per cm^3 of column volume. In accord with the results of GLAZER AND WELLNER[1] the binding was found to be irreversible as long as the buffer was not changed: exhaustive washing failed to elute any protein, and bound ^{14}C-labelled protein (partially reduced[3] and alkylated with ^{14}C-iodoacetamide) did not exchange with unlabelled protein put through

subsequently. It appears therefore that application of sufficient protein in the first run of a series will result in the binding sites being saturated and unavailable during the following runs.

Results similar to those in Fig. 1 were obtained when immunoglobulin heavy chains (40 mg) or hen ovalbumin (60 mg) were put through the same column in the same buffer. The capacity of the column for binding the latter was only about 0.01 mg per cm^3, but the disparity between the amounts of monomer and aggregate bound led to the aggregate being assessed as 11% of the total protein in the first run and 19% in subsequent runs.

Consider a protein present in solution in both monomeric and aggregated forms, not interconverting, passed through a column of dextran gel in which the former is incompletely and the latter completely excluded from the gel phase. The aggregate will move faster through the column, will be the first to encounter those binding sites accessible externally on the gel grains, and may saturate the sites so that they are unavailable to the monomer. Presumably the monomer will now encounter sites in the interior of the grains which were inaccessible to the aggregate. But there is clearly no guarantee that the proportions bound will be the same for each species. In the examples given the aggregate was always bound in greater proportion, but a reverse situation is conceivable.

The simplest way to avoid the error described is to put repeated aliquots through a single column, with no intervening change of buffer, until the elution profiles given in two successive runs are the same. We have found this procedure much more reliable than mere estimation of recoveries. In practice it need only imply a single priming application of protein to enable a column to be used repeatedly for assessing dispersity in different samples.

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