COMPARISON OF THE EFFECTS OF VARIOUS DETERGENTS ON ANTIGEN–ANTIBODY INTERACTION

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1. Introduction

Sodium dodecyl sulphate solubilizes and dissociates a large variety of macromolecular complexes [1]. Antigen–antibody precipitates are dissolved at concentrations of 0.5% [2, 3] and at lower concentrations (0.2%) the precipitation of ovalbumin by antibody is completely inhibited [4]. Some other detergents, although effectively solubilizing complexes such as biological membranes, do not apparently inhibit antigen–antibody precipitation (e.g. 1% sodium deoxycholate [4, 5], 1% Triton X-100 [6] and 0.5% Nonidet P-40 [7, 8]). If precipitation persists in the presence of detergent, then specific antisera can be employed to selectively remove and purify components from detergent-solubilized mixtures. The present paper compares the effects of various detergents on the precipitation of a number of antigens by their homologous antisera. Detergents were selected primarily on the basis of their current use as solubilizing agents. The results indicate that antigen–antibody precipitation was markedly inhibited by 0.5% sodium dodecyl sulphate and Sarkosyl-L, but 1% sodium deoxycholate, Brij 58, Tween 80, Triton X-100 and Nonidet P-40 had a negligible effect.

2. Materials and methods

Detergents were obtained from the following sources and were used without further purification: sodium deoxycholate and Triton X-100 (*p*-octylphenoxy-polyoxyethylene) were from Sigma (London) Chemical Co. Ltd., sodium dodecyl sulphate (especially pure) was from BDH Chemicals Ltd., Nonidet P-40 (alkyl phenyl ethoxylate) was from Shell Chemicals Ltd., Brij 58 (polyoxyethylene cetyl ether) was from Atlas Chemical Corp., Tween 80 (polyoxyethylene sorbitan mono-oleate) was from Koch-Light Laboratories Ltd. and Sarkosyl L (sodium dodecyl sarcosinate) was donated by Geigy (U.K.) Ltd.

Sperm-whale metmyoglobin [9], and mouse IgM [10], IgG2a [11] and urinary light (λ) chain [12] were prepared as previously described. Radioactive samples of the above mouse proteins were isolated from the culture supernatants of myeloma cells that had been incubated for 4–6 hr with L-[4, 5-^{3}H] leucine [10, 11]. Antisera were raised in rabbits [9, 10].

The effect of detergent on antigen–antibody precipitation was measured using amounts of antigen and homologous antiserum that were at equivalence in the absence of detergent. Mixtures of antigen, antibody and detergent were incubated at 37° for 1 hr and subsequently at 2° overnight with the exception of those containing sodium dodecyl sulphate which were left overnight at room temp. Antigen–antibody precipitation in the presence of sodium deoxycholate and Sarkosyl L was carried out using 75 mM-Tris-HCl buffer–75 mM NaCl, pH 8.1 (Tris buffer–saline) as solvent, whereas 10 mM sodium phosphate buffer–130 mM NaCl–4 mM KCl, pH 7.4 (phosphate buffer–saline) was used with the other detergents; solutions of sodium deoxycholate (1%) in phosphate buffer–saline (pH 7.4) formed a gel on standing at 2°due to the presence of deoxycholic acid. Amounts of immune precipitate were estimated either spectrophotometrically [9] or by radioactive assay [12].

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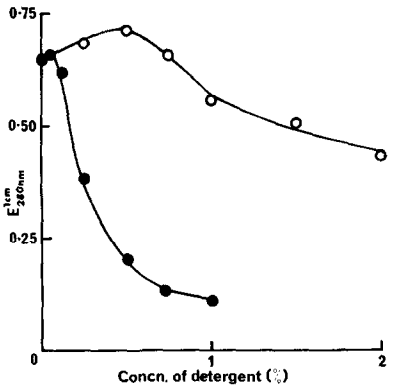

Fig. 1. Effect of increasing concentrations of sodium deoxycholate and Sarkosyl L on the amount of metmyoglobin–antibody precipitate. Metmyoglobin (22 μg) was added to increasing amounts of either a 10% (w/v) solution of sodium deoxycholate (o–o–o) or a 4% solution of Sarkosyl L (•–•–•) and the volume adjusted to 0.25 ml with Tris buffer–saline. Anti-(metmyoglobin) serum (0.25 ml) was then added; this amount of antiserum gave maximal precipitation of the antigen in the absence of detergent. The washed precipitates were dissolved in 0.6 ml of 0.1 M NaOH and the extinction at 280 nm of the solution measured.

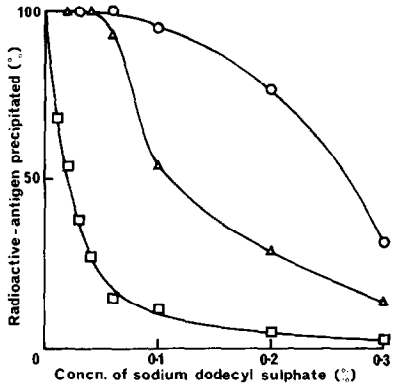

Fig. 2. Effect of increasing concentrations of sodium dodecyl sulphate on the precipitation of mouse IgM by anti-(IgM) serum (o–o–o), mouse IgG2a by anti-(IgG) serum (Δ–Δ–Δ) and mouse light chain by anti-(light chain) serum (□–□–□). Equivalent amounts of the radioactive antigens were mixed with their homologous antisera in the present of increasing amounts of the detergent. The radioactive precipitates were washed and counted as previously described [12].

3. Results

Concentrations within the range of 0–2% (w/v) of Tween 80, Triton X-100, Brij 58 and Nonidet P-40 had no detectable effect on the precipitation of radioactive mouse IgM, IgG2a and light chain by their homologous antisera. The effect of these detergents at concentrations higher than 2% was not investigated.

Fig. 1 shows that increasing concentrations of sodium deoxycholate caused a small initial increase and then a gradual decrease in the amount of precipitate formed by sperm-whale metmyoglobin with an anti-(metmyoglobin) serum; 1% (w/v) sodium deoxycholate gave 14% inhibition of precipitation. Similar results have been obtained with different antisera to metmyoglobin, and with different antigen–antibody systems (e.g. pig IgG, and mouse IgM, IgG2a and light chain with their respective antisera). In each case, the inhibition of precipitation caused by 1% (w/v) sodium deoxycholate was within the range 0–14%. In contrast to sodium deoxycholate, Sarkosyl L and sodium dodecyl sulphate had a very much more marked inhibitory effect. For example, 0.25% Sarkosyl L caused a 50% decrease in the precipitation of metmyoglobin (fig. 1). A similar relationship between detergent concentration and inhibition of precipitation was established for sodium dodecyl sulphate using mouse IgM as the antigen (fig. 2). On the other hand, 50% inhibition of precipitation of mouse IgG and light chain occurred at lower concentrations of sodium dodecyl sulphate (0.1 and 0.02%, respectively; fig. 2). The reason for the variation in the amount of detergent required for the same degree of inhibition with different antigens is not known. It may, however, be related to the difference in molecular size and/or carbohydrate content of IgM, IgG and light chain.

4. Discussion

The present results suggest that detergents may be divided into two groups on the basis of their capacities to inhibit antigen–antibody precipitation; namely those which at a concentration of 0.5% or less profoundly inhibit precipitation and those which at a concentration of 1% have a small or no detectable effect.

A decrease in precipitation in the presence of detergent may be due to a covering-up of combining-sites by bound detergent, a change in conformation of the reactants or the disruption of noncovalent forces. Sodium dodecyl sulphate has been shown to bind strongly and to induce conformational changes in proteins [13]. Its marked inhibition of precipitation is undoubtedly related to these effects and is consistent with its capacity to dissociate macromolecular protein complexes [1]. Sarkosyl L also dissociates protein complexes [14, 15] and its inhibitory activity probably has a similar basis to that of sodium dodecyl sulphate. Cationic detergents with long alkyl chains (e.g. cetylpyridinium chloride) have been shown to induce conformational changes in proteins [16] and, consequently should also markedly inhibit precipitation. On the other hand, detergents having a negligible effect on precipitation probably do not cause conformational changes and furthermore, if the detergent is bound, then the strength of binding is low relative to the strength of antigen–antibody interaction.

Why do detergents differ so markedly in their effects? It may be significant that the detergents having little effect on antigen–antibody precipitation were either non-ionic or, in the case of sodium deoxycholate, the salt of a very weak acid (pK 6.58), whereas those having a profound effect were the salts of stronger acids and contained long flexible hydrocarbon chains. Presumably ionic detergents are bound to proteins via salt linkages. Further, it seems likely that flexible hydrocarbon chains fit more readily than more rigid bulky groups (e.g. deoxycholate) into the nonpolar crevices of the surfaces of proteins, and consequently are bound more strongly. (It has been calculated [17] that nonpolar atoms occupy 40–50% of the surface area of globular proteins). However, although the degree of binding of ionic detergents and the induction of conformational changes depends on the length of the hydrocarbon chain [16, 18], it appears that inhibition of precipitation is predominantly due to the effects of charge repulsion since the majority of the non-ionic detergents examined also possessed long hydrocarbon chains. Thus, binding of an ionic detergent converts proteins into polyions with consequent disruption of conformation and abrogation of intermolecular interaction.

The practical relevance of these results is that proteins solubilized by non-ionic and certain other detergents may be isolated using specific antibodies (e.g. affinity chromatography). Furthermore, our experience with sodium dodecyl sulphate suggests that even this detergent may be used providing the antigen possesses a suitable size and/or composition.

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