The reactions between glutaraldehyde and various proteins. 
An investigation of their kinetics

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Synopsis. Glutaraldehyde reacts readily with various proteins in solution. With high concentrations of both, the solutions become yellow and many proteins form a gel. At low concentrations the reactions may be followed by the changes in the u.v. spectrum between 250 and 300 nm. The reverse reaction does not proceed to any detectable extent. The kinetics are pseudo-first-order. The activation energies for the reactions between proteins and glutaraldehyde were found to be about 11 kcal/mole. This suggests that the proteins have not been denatured to any marked extent by the glutaraldehyde fixation. The rates of reactions increase with pH. The rate of formation of glutaraldehyde-protein links per protein molecule glutarated is approximately 1 sec⁻¹ mol⁻¹ l⁻¹.

Introduction

Glutaraldehyde is a fixative much used for electron microscopy. The chemical processes involved, however, are little understood. Previously, work on glutaraldehyde has been largely concerned with its immediate practical properties, e.g. on the preparation of tissues for electron microscopy (Sabatini, Bensch & Barnett, 1963). There is now a growing literature on various aspects of glutaraldehyde such as purification (Fahimi & Drochmans, 1965; Anderson, 1967), rate of penetration into tissues (Hopwood, 1967a; Ericsson & Biberfeld, 1967; Chambers, Bowling & Grimley, 1968), effects on various enzymes (Janigan, 1964, 1965; Quiocio & Richards, 1966; Hopwood, 1967a), behaviour on the gel Sephadex G-10 (Hopwood, 1967b), comparison of its crosslinking abilities with those of formaldehyde and α-hydroxyadipaldehyde (Hopwood, 1969a), and use in the tanning industry (Bowes & Cater, 1965, 1968). A more complete review has been written by Hopwood (1969b).

It has been assumed previously, by analogy with formaldehyde, that glutaraldehyde reacts
with the primary amine groups of basic amino acid residues in proteins. Bowes & Cater (1965) have shown that apparently only lysine amongst the basic amino acids in proteins combines with glutaraldehyde. The effect on aromatic amino acid residues has been investigated more recently (Bowes & Cater, 1968). The present communication is concerned with an investigation into the overall average kinetics of the reaction between glutaraldehyde and various proteins.

A preliminary account of this work has been presented elsewhere (Hopwood, 1968).

Materials and methods

Glutaraldehyde was obtained from Union Carbide Ltd (London, W.I) and was purified by adsorption with activated charcoal (Anderson, 1967). The concentration of glutaraldehyde was determined titrimetrically (Clift & Cook, 1932). The proteins investigated were bovine serum albumin, casein, acid phosphatase (wheat germ), x-chymotrypsinogen, fibrinogen (human), haemoglobin (bovine), IgG (y-globulin), lysozyme, ovalbumin, papain and protamine. They were obtained from British Drug Houses Ltd or Sigma Chemical Co.

The qualitative effects of 4% glutaraldehyde on the above proteins were determined at pH 7.2 in the presence of 0.1 M phosphate buffer. The protein solutions used were 2%. Colour changes and gel formation were noted.

The changes in u.v. spectrum between 250 and 300 nm, associated with the reactions between glutaraldehyde and bovine serum albumin, casein and acid phosphatase were followed with a Unicam SP 800 recording spectrophotometer. This was fitted with a constant temperature cell housing attached to a thermostatically controlled water bath; the reagents were placed in this water bath to allow their temperatures to equilibrate before the reactions were run. The reactions were carried out in a cuvette containing an excess of glutaraldehyde, 1 or 2 mg of protein and 0.03 M phosphate buffer at pH 7.2. Spectra were run between 250 and 300 nm at minute or 30 sec intervals. The effect of glutaraldehyde on phenylalanine, histidine, tyrosine and tryptophan at pH 7.2 was investigated similarly.

The reversibility of the reactions was investigated by allowing a glutaraldehyde–protein reaction mixture at pH 7.2 to stand overnight in order to come to equilibrium. The spectrum was run and aliquots of the mixture diluted by various amounts. Their spectra were run at intervals over the next 24 hr and a concentration–optical density curve plotted.

The effect of pH on the reactions was determined by carrying out runs at a constant temperature at pH values in the range 5.5–9.0 in the presence of 0.03 M acetate, phosphate and borate buffers.

The effect of temperature on the reaction rates was determined by carrying out runs at pH 7.2 at various temperatures in the range 15–40°C.

Results

Reaction between glutaraldehyde and proteins

Concentrated protein solutions reacted with glutaraldehyde to give a yellow colour, unless the protein was initially pigmented. The depth of colour increased with time. Some proteins formed a gel with glutaraldehyde within 15–30 sec, whereas others did not, even over 24 hr. In the experiments with dilute proteins only lysozyme gave a precipitate. The others became very pale yellow over a 24 hr period. The results of these experiments are summarized in Table 1.