Antigen-Antibody Interactions

Conformational Changes in Protein Antigens Induced by Specific Antibodies: Sperm-whale Myoglobin

M. J. CRUMPTON

National Institute for Medical Research, Mill Hill, London NW7 1AA, Great Britain

With 7 Figures

Introduction

It is well recognized that the conformations of globular proteins are particularly sensitive to environmental changes and that they may be altered by the binding of specific ligands. As a result, it seems likely that interaction between proteins or between the polypeptide chains of multisubunit proteins will also be associated with localized or more extensive conformational alterations.

Antigen-antibody interaction is one system in which attempts have been made to correlate specific binding with changes in the conformations of the reactants. However, contrary to some claims, investigations of the effect of the antigen on the conformation of the antibody have failed to provide convincing evidence in support of the view that binding of antigen by the antibody combining-site causes conformational changes elsewhere in the antibody molecule (for a critical review of the evidence see METZGER, 1970). This interpretation of the data is supported by the results of more recent studies of the effect of bound ligand on the circular dichroism spectra, the rate of exchange of labile hydrogens and the susceptibility to proteolysis of a homogeneous human macroglobulin [ASHMAN et al., 1971; ASHMAN and METZGER, 1971]. In contrast, data have been presented by a number of investigators suggesting that specific antibodies may either induce a conformational change in globular protein antigens or, alternatively, select and stabilize a particular conformation of the antigen. For example, mutants producing defective enzymes have been activated to full enzymic
activity by antibodies to the wild-type enzyme [penicillinase; Pollock et al (1967); β-D-galactosidase; Rotman and Celada (1968); Melchers and Messer (1970); catalase, Feinstein et al. (1971)]. Similarly, the restoration of the activity of heat-denatured acetylcholinesterase by antibodies to the native enzyme [Michaeli et al., 1967] and the enhancement of enzymic activity by certain homologous antisera [cephalosporin β-lactamase, Chong and Goldner (1970); ribonuclease, Cinader et al. (1971); glutamate dehydrogenase, Lehmann (1971)] has been accounted for in terms of a modification of the conformation of the antigen. Conformational changes are most probably also the cause for the observations that form the basis of this report; namely, the release of ferrihaem from sperm-whale metmyoglobin by antibodies to apomyoglobin (i.e. the haem-free protein). An account of this phenomenon has been published previously [Crumpton, 1966].

Comparison of Metmyoglobin and Apomyoglobin

A molecule of sperm-whale myoglobin is composed of one polypeptide chain containing 153 amino acid residues; no cyst(e)ine is present [Edmundson, 1965]. X-ray crystallographic analysis [Kendrew et al., 1961] of the oxidised protein (metmyoglobin) showed that the amino acid residues are arranged in helical segments which are separated by non-helical regions and that the polypeptide chain is folded into a bag-like structure containing a non-polar pocket which is occupied by the haem group (Fig. 1).

It is generally accepted that the structure of metmyoglobin in solution resembles closely that of the crystalline protein [Stryer, 1968; Hugli and Gurd, 1970 (1), (2)]. Evidence has, however, been presented in support of the view that the conformation in solution is more motile than in the crystal [Stryer, 1968; Hugli and Gurd, 1970 (2)], and that dissolved proteins are in a state of dynamic equilibrium with localized unfolding constantly occurring [Schechter et al., 1968]. For example, difference Fourier studies of metmyoglobin and azide-metmyoglobin showed no detectable change in the conformation of the protein when the azide ion is bound at the sixth coordination position of the haem [Stryer et al., 1964]. However, if the conformation of the protein was fixed, azide would not be bound because access to the haem is blocked by neigh-