IMMUNOLOGY

Immobilization Antigen in Heterozygous Clones of *Paramecium aurelia*

CLONES of *Paramecium aurelia*, heterozygous for two alleles at one of the loci specifying the immobilization antigens[1], react to both parental type-specific antisera, but usually more strongly to one parental type than to the other. It is of considerable interest to determine whether these heterozygous antigens consist of one molecular species combining in varying degree the specific regions of the parental protein molecules or whether there is simply a mixture of the parental antigens. On the basis of refined serological tests, Finger and Heller[2] have suggested that a single hybrid molecular species is present and that the variation between clones (sometimes even derived from the same mating) is due to the formation of a hybrid combining in different ways the characters and presumably primary structure of the parental proteins.

Animals of stocks 60 and 90 of *P. aurelia* variety 1, expressing the D serotypes, were mated to produce clones of phenotype 60D/90D. About 10^9 of these cells were grown and the immobilization antigen extracted by methods which have been described elsewhere[5]. The antigen was freed from most of the contaminating protein by passing through a column of '*SE*-Sephadex' with a *p*H gradient between *p*H 4·2 and *p*H 5·6 in 0·05 *M* sodium acetate. This treatment produces no separation of any of the immobilization antigens which have been examined. 60 mg of antigen was then fractionated on a column of hydroxyapatite[3,4] at *p*H 6·8 at 2°–3°. Elution was with a series of sodium phosphate buffers 0·002 M, 0·010 M, 0·25 M and 0·050 M applied by a constant rate pump at 3–4 ml./h. Some 100–150 ml. of each buffer was used. Fig. 1 shows the optical density at 280 mμ. The fractions were also examined for their capacity to inhibit the immobilization reaction between both 60D animals and anti-60D serum and 90D animals and anti-90D serum (Table 1).

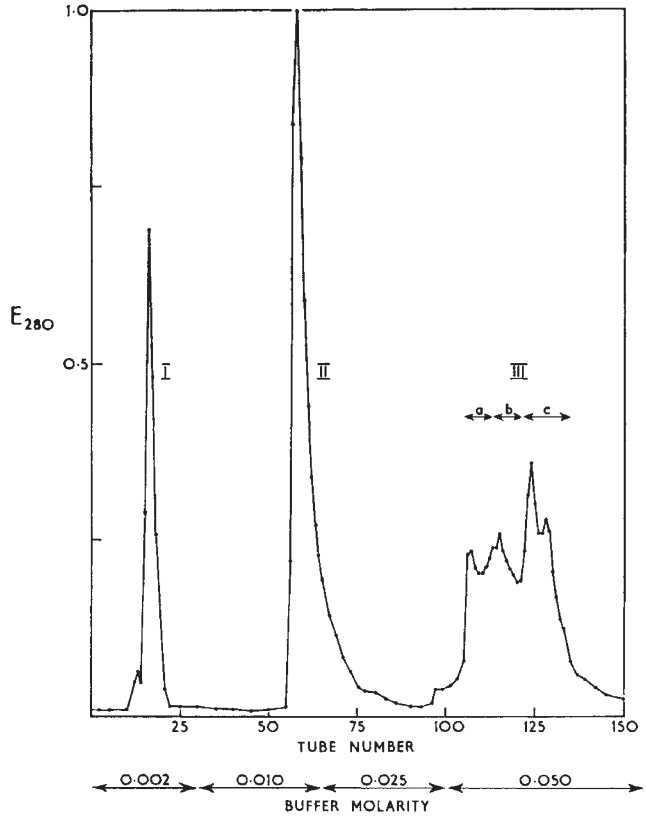

Fig. 1. Fractionation of 60/90D antigen on hydroxyapatite

Table 1

Fraction	Inhibition of immobilization reaction 60D	90D
I	–	–
II	+ + + +	–
III *a*	+ + +	+
b	+ +	+ +
c	+	+ + +

Peak I is completely inactive and is thus the remaining impurity. Peak II is eluted in the position characteristic of pure 60D antigen and gives no reaction for 90D. Pure 90D antigen would normally be eluted in the region III*b*–III*c*. Fractions III*a* and III*c* were each refractionated under the same conditions and were again eluted in the same region and showed the same activity pattern. No elution in any other region of the chromatogram was observed.

Thus the heterozygote antigen contains parental 60D material amounting to about 50 per cent of the total antigen. However, the remainder reacts with both anti-60D and anti-90D antisera, and consists of at least two molecular species. It was not possible to determine whether any pure 90D was present because the resolution in this region was not sufficiently good. When the original heterozygote antigen was examined serologically it appeared to contain 70–80 per cent 60D activity.

Other preparations of 60/90D antigen have given similar results.

The immobilization antigen is a protein of molecular weight 250,000 and contains two identical half-molecules held together by many disulphide bonds. These half-molecules in turn probably consist of several non-identical sub-units[5]. In the heterozygote the molecule might be constructed in two ways: (1) The half-molecules, made independently to give two species $(60D)_{\frac{1}{2}}$ and $(90D)_{\frac{1}{2}}$, might then combine randomly to give three possible species—60D, 90D and $(60D)_{\frac{1}{2}}(90D)_{\frac{1}{2}}$. In the present experiment where 60D comprises 50 per cent of the total, 90D would be 10 per cent and the hybrid 40 per cent. (2) The half-molecules might be randomly assembled from the possible sub-units, and then form dimers. In the simplest case of only two sub-units, say $(AB)_2$, the species formed would be $(A^{60}B^{60})_2$, $(A^{90}B^{90})_2$, $(A^{60}B^{90})_2$ and $(A^{90}B^{60})_2$ and the amounts in this instance 50, 10, 20 and 20 per cent. In both cases the total amount of 60D activity would be 70 per cent of the whole. Either of these simple hypotheses, particularly the second, can explain the results but, of course, it may be that the situation is more complex. Implicit in the second scheme is the suggestion that the sub-units are specified by separate genetic loci. Beale[6] was unable to demonstrate recombination between alleles at the D locus, but the resolving power of his methods was rather low and close linkage cannot be ruled out.

These results differ basically from those of Finger and Heller[2], who suggested that only one species was formed in any clone and that the observed variation in the degree of parental type reaction was not a consequence of quantitative variation but of the exclusive formation of different hybrids in different clones. The present results suggest rather that the quantitative variation is basic and that hybrids arise in a purely random way. This quantitative variation will be examined more thoroughly. It can be considered as partial dominance at the molecular level or, in the broader sense, as an example of a very simple form of differentiation.

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