Nature of the Binding

In order for binding to have been detected under the experimental conditions used, the repressor must bind very tightly to DNA. A close examination of Figs. 2 and 3 shows that some of the bound repressor washes off the DNA as it sediments. This suggests that the dissociation constant is of the same order of magnitude as the concentration of DNA binding sites (called operators) in the peak tubes. Assuming a small number of operators per phage genome (there are probably one or two), this value is roughly 10^{-9}–10^{-10} M. A repressor-operator affinity in this range *in vivo* is suggested by the magnitude of derepression observed with the *lac* operon. Since a 1,000-fold increase in β-galactosidase synthesis occurs on induction, the dissociation constant of the repressor–operator complex should be 1,000-fold less than the concentration of free repressor in the cell[8]. The concentration of free *lac* repressor has been estimated at 10^{-7} M (ref. 2), implying that the dissociation constant is of the order of 10^{-10} M.

The finding of the λ*ind*⁻ repressor to λ DNA was noticeably weaker when the complex was sedimented through a sucrose gradient containing 0·1 M KCl instead of the 0·05 M KCl used in the experiments described here. In a gradient containing 0·15 M KCl, no binding was detected. This observation suggests that the binding is partly electrostatic.

The finding that the λ repressor binds specifically and with high affinity to λ DNA strongly suggests that the simplest model for the mechanism of action of the repressor is correct—namely, that the repressor blocks transcription from DNA to RNA by directly binding to DNA. This conclusion is further supported by the recent observation of Dr. W. Gilbert that the *lac* repressor binds specifically to *lac* DNA and is removed by IPTG (W. Gilbert, to be published).

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Tertiary Structure of Ribonuclease

by

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Two models have recently been proposed for the tertiary molecular structure of ribonuclease (*Nature*, 213, 557 and 862; 1967). The models were based, respectively, on 5·5 Å and 2 Å electron density maps built up over the past decade and a half. An attempt is here made by a proponent of one of the models to reconcile the considerable differences that exist between the two models.

A MODEL has been proposed from this laboratory for the three-dimensional folding of the polypeptide chain in bovine pancreatic ribonuclease[1]. A little earlier, Avey *et al.*[2] had proposed another model of the same protein in the same crystalline form, and in a note appended to that article Dr. C. H. Carlisle had commented on some apparently significant differences between that model and ours.

The purpose of the present article is to point out that in my opinion the disagreement is probably not in the data or the results but in the interpretation of the separate electron density maps computed by the two groups with very different volumes of X-ray diffraction data as input. To be specific: our map was computed at 2 Å resolution with 7,294 reflexions, whereas Avey *et al.* (hereinafter called the London group) used 389 reflexions within 5·5 Å resolution in building their map. To this extent, the two maps which were the starting point for the two separate models are widely different. To indicate the effect of resolution on the quality of the map, three maps are given in Figs. 1–3. Fig. 1 was computed with the 2 Å data of 7,294 reflexions and contours are drawn at intervals of 200 units on an arbitrary scale; the first dotted contour corresponds to 100 units. Figs. 2 and 3 were also computed with our data, but terminated at 5·5 Å and included 362 reflexions to correspond to the data range used by the London group. In Fig. 2 contours are drawn in the same intervals as in Fig. 1; however, in Fig. 3 contours are in intervals of 40 units, starting the dotted with 20. The maps are *xy* sections corresponding to $z = 0{\cdot}357$, this being the co-ordinate of the disulphide bridge we have designated as II–VII. This bridge is denoted in each map by a cross.

In Fig. 1, this point corresponds to a peak density of 1,700 and is one of the five highest peaks in half the unit cell. In Figs. 2 and 3, the peak height at the corresponding point is 120 on the same unit. There are fifteen peaks of height greater than 150 in this map including two regions of peak height of more than 200 units. The first of these corresponds to the region we have identified as in the vicinity of the bulky residues GLN(28), MET(29), and MET(30). The second highest region in this 5·5 Å corresponds to the possibly helical region of the main chain residues 52–58. From a detailed comparison of this 5·5 Å resolution map with 4·0, 3·0, 2·4, and 2·0 Å resolution maps, I conclude that at the 5·5 Å resolution, the most prominent features of the electron density map do not correspond to the disulphide bridges. In the 4 Å maps these bridges do become visible, but one has to go to a resolution of at least, if not better than, 3 Å before the disulphides stand out as the prominent features.

The co-ordinates of the centre of the disulphide bridges obtained from our 2 Å maps are given in Table 1 with corresponding co-ordinates of the London group. We believe our co-ordinates to be accurate to 1 Å. Comparison of these co-ordinates with those of the London group show no direct correspondence even allowing for the possibility of different choice of origins. This is true even for the first of these two bridges, and thus it seems likely that the cystine co-ordinates as listed by the London group differ considerably from our set. Furthermore, I think that a detailed interpretation of the main chain folding in a protein of low helical content from a low resolution map on the assumption that the most prominent features are indeed the disulphide bridges is an extremely

(*Continued on page 330*)

(*Continued from page 234*)

difficult and hazardous, not to say impossible, task without very specific outside information.

On the other hand, from a 5·5 Å resolution map, it is possible to locate the heavy atom positions and binding sites (ref. 3 and unpublished results) and I think that with the proper change of origin, the region of the active site as found by the two groups can be brought into reasonable proximity. In fact, even though this position is nowhere specifically given, an investigation of their heavy atom positions suggests that the two origins are related by an $a/2$ and $c/2$ translation along x and z axes, and approximately the same origin along the y axis, but with a reversal of its direction. With this correlation of origins, it is possible to identify the regions of the molecule in our map that have been designated as disulphide bridges by the London group and it is seen that these regions are appreciably different from the cystine positions given here.

Table 1. POSITION OF THE DISULPHIDE BRIDGES OF RNase ACCORDING TO THE 2 Å MAP AND TO AVEY *et al.*[2]

Disulphide bridge	Co-ordinate set (*A*)*			Co-ordinate set (*B*)*		
	x/a	*y/b*	*z/c*	*x/a*	*y/b*	*z/c*
I–VI	0·48	0·24	0·15	0·69	0·45	0·09
II–VII	0·73	0·10	0·36	0·22	0·85	0·45
III–VIII	0·25	0·76	0·32	0·00	0·75	0·14
IV–V	0·13	0·59	0·39	0·94	0·45	0·46

* (*A*) According to the 2 Å map; (*B*) according to Avey *et al.*[2].

Thus, in summary, while with proper changes of origin the disagreement about the region of active site established by the London group can be reconciled with that which we found from the phosphate binding site, the disulphide positions as found from the 5·5 Å map by them are probably incorrect and their model, which is largely based on the assumption of the correct location and designation of these bridges, may need extensive modifications. It seems likely, however, that when adequate X-ray data are used in computing the electron density map by Avey *et al.*[2], it will be possible to construct from it a model which is more objective and less dependent on the correct identification of the S–S bridges alone; and that model will not differ much in its main features from the one which we propose.

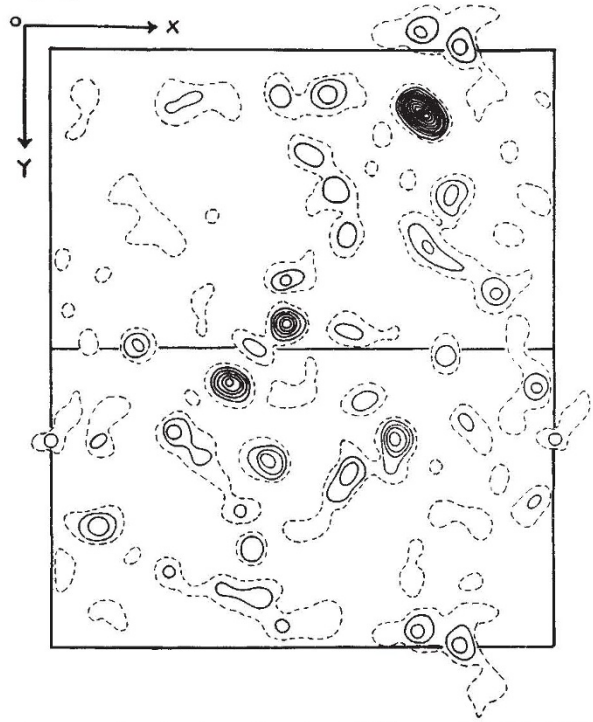
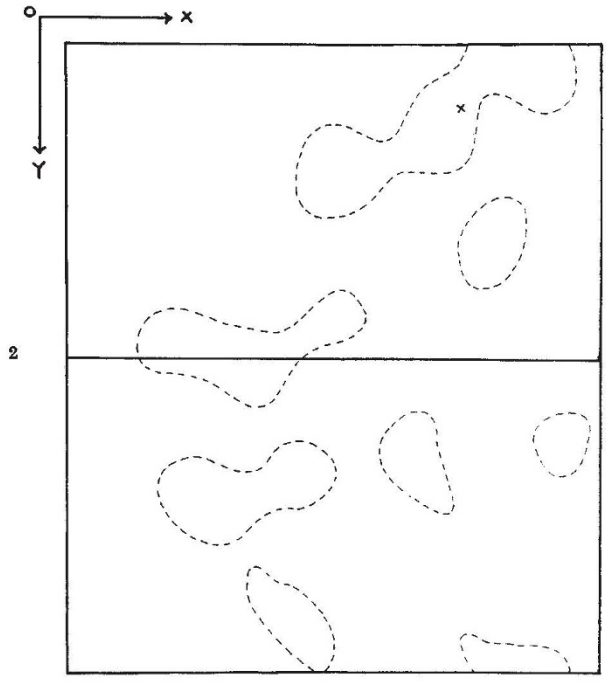
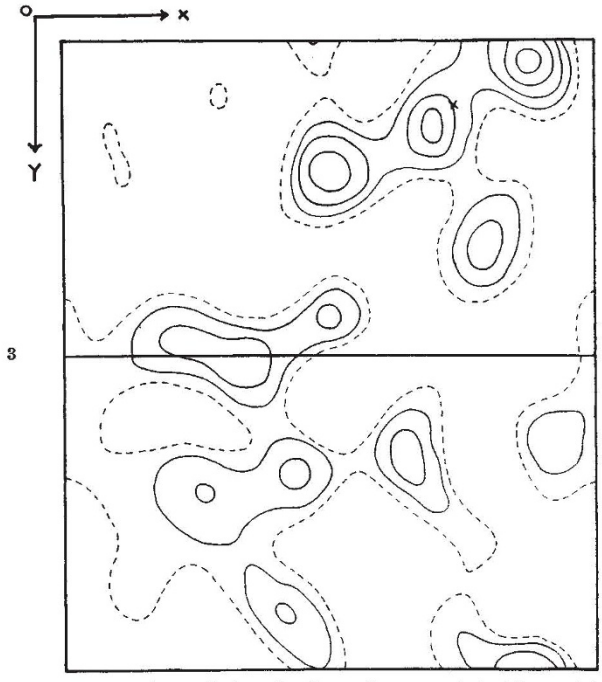


Fig. 1. Electron density section at $z = 0{\cdot}357$ from a map computed at 2 Å resolution and 7,294 reflexions. Contours at 200 units interval. Dotted contour 100.





Figs. 2 and 3. Same electron density section computed with our data terminated at 5·5 Å. This involved 362 reflexions. In Fig. 2, the contours are the same as in Fig. 1, and in Fig. 3, dotted contour corresponds to 20 and remaining contours are in steps of 40.

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