STEREOCHEMISTRY OF THE PUROMYCIN REACTION

by

I. D. Raacke

Department of Biology, Boston University, Boston, Massachusetts 02215

Received February 22, 1971

SUMMARY

Space-filling models have shown that peptidyl-puromycin cannot be formed if puromycin is displacing the end of aminoacyl-tRNA. Instead, the models show that puromycin "hugs" the terminus of peptidyl-tRNA in such a way that the free amino group of the antibiotic is in a perfect position for a nucleophylic attack on the carbonyl carbon of the peptide. In this configuration there is a continuous "stack" of four hydrophobic rings, with the dimethyl-adenine and methoxy-benzene of the antibiotic alternating with the terminal adenine and penultimate cytosine of peptidyl-tRNA. This is an essential feature because it helps to bind the tRNA after its adenine has been displaced from the peptidyl site. The model predicts the existence of a puromycin-binding protein in the ribosome.

It is well known that puromycin is an analogue of the aminoacyl-tRNA terminus (1), and it is universally assumed that it takes the place of aminoacyl-tRNA and thereby becomes the "acceptor" of the growing peptide chains (2,3). In terms of the translocation model for protein synthesis, this means that the puromycin must come to occupy the aminoacyl site on the 50S ribosome and the peptidyl-tRNA must be in the peptidyl site (4,5).

One difficulty with this view is that there seems to be only one site on the 50S subunit capable of recognizing, i.e. binding the terminal adenosine of tRNA, and that site seems to be occupied by peptidyl-tRNA (6-8). It is quite possible therefore, that when the aa-tRNA is in the A site, its adenosyl end is not bound on the 50S ribosome--and hence there would be no corresponding site for the cognate dimethyl adenine of the puromycin to bind (8). Furthermore, it has been shown that while puromycin liberates the tRNA bound to a peptide, it does not alter the amount of ribosome-bound aa-tRNA (9); and neither is the binding of the latter inhibited by the antibiotic (6). All of this strongly indicates that puromycin does not displace the aa-tRNA from the A site prior to or as a result of its reaction with the growing peptide chain.

A further and more serious difficulty to the view that puromycin acts as an analogue of aa-tRNA in peptide bond formation is that by working with Corey-Pauling-Koltun atomic space-filling models of the antibiotic and of the peptidyl-tRNA terminus, I found that if a puromycin molecule and a tRNA were laid side by side and facing in the same direction, it is impossible to make the free amino group of puromycin reach the carbonyl group of the peptide--as it must do if reaction between the two is to take place.

I also found, however, that the puromycin molecule can assume a U-shaped conformation, with the benzene ring stacked under the dimethyladenine--a conformation which was recently proven to be correct by X-ray crystallography (10). In this configuration the antibiotic can "hug" the terminal adenine of peptidyl-tRNA in such a fashion that its own free amino group is in a sterically perfect position to make a nucleophylic attack on the carbonyl group of the peptide. Furthermore, the dimethyladenine of puromycin, the terminal adenine, the methoxy-benzene of the antibiotic, and the penultimate cytosine of tRNA form one continuous "stack" of hydrophobic rings. The interaction of space-filling models of puromycin and peptidyl-adenosine (alanine is used instead of the peptide to keep the model simpler) is represented in Fig. 1 in

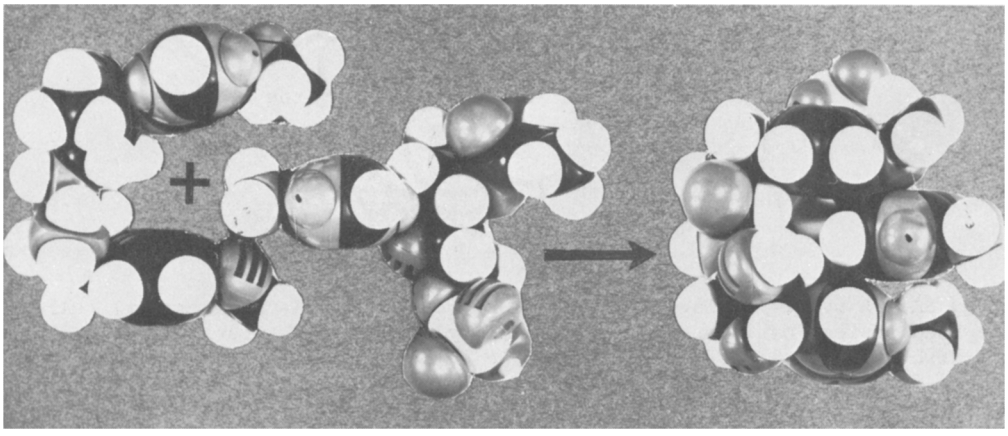

Fig. 1. Representation of the reaction of puromycin with peptidyl-adenylic acid (to represent the end of peptidyl-tRNA) by means of Corey-Pauling-Koltun space-filling models, showing the stacking of the dimethyladenine, adenine and methoxy-benzene, respectively, in the intermediate reaction complex.

such a way as to show the fit of three of the ring systems. Fig. 2 represents the other side of the alanyl-tRNA-puromycin complex and shows how the amino group of the antibiotic attacks the carbonyl group of the "peptide" in the puromycin reaction.

The mechanism suggested by the sterical models confirms that there is no interaction of the puromycin with the aminoacyl site and that all interactions are confined to the peptidyl site. Since the adenine of peptidyl-tRNA is bound to the peptidyl site (6-8), there must be a protein or a locus formed by several proteins capable of binding adenine. When the puromycin "stacks" its dimethyladenine on the adenine, it must interpose itself between the adenine and its binding site, and thus become bound itself. The peptidyl-tRNA

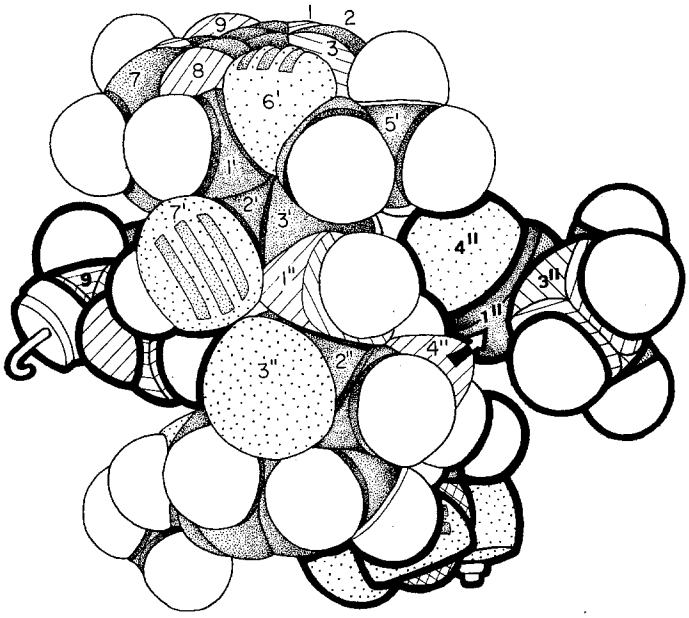

Fig. 2A. A space-filling model of a puromycin-peptidyl-tRNA complex. (Alanine is used instead of a peptide for simplicity's sake). The puromycin is shown in light outline and the alanyl-adenylic acid representation of the 3' OH terminus of tRNA in heavy outline. The arrow shows the position of the free amino group of the antibiotic ready to execute a nucleophylic attack on the carbonyl carbon of peptidyl-tRNA to yield peptidyl(alanyl)-puromycin.

The white atoms are H (with the hook representing a H bond); the heavily stippled ones, C; the lightly stippled ones, double-bonded O, and the same with slits, single-bonded O; the slatted atoms are N, and the cross-hatched one at the bottom, P.

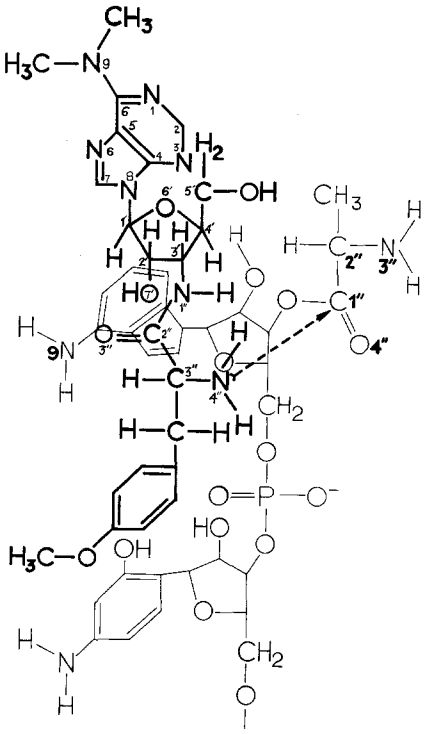

Fig. 2B. Representation of the model in Fig. 2A by means of chemical formulae, except that alanyl-adenylyl-cytidylic acid is used to represent the terminus of peptidyl-tRNA. The purpose of the numbering system is to correlate the formulae with the atoms in the model.

---

then remains bound by the hydrophobic attraction between the stacked bases. This explains the hitherto unexplained fact that the aromatic character of the amino acid in puromycin is indispensible for activity (11,12), for if there were no second ring in the antibiotic, the peptidyl-tRNA could not remain bound once its adenine had been displaced from its binding site. The actual existence of a puromycin-binding protein in ribosomes has been demonstrated in this laboratory and will be reported separately.

For the puromycin reaction to occur there must be a transfer of the peptidyl group onto an amino group acceptor, just as in the case of peptide bond formation. It seems likely, therefore, that the protein capable of binding the adenine of peptidyl-tRNA as well as the dimethyladenine of puromycin is a peptidyl transferase. I have shown, however, by other space-filling models

to be reported elsewhere, that the amino group of an aa-tRNA during protein synthesis and the amino group of puromycin must approach the donor carbonyl group of the peptidyl-tRNA from opposite directions. It remains an open question, therefore, whether the puromycin reaction and the formation of peptide bonds are exactly equivalent, or if they are even catalyzed by the same peptidyl transferase.

Another interesting aspect of the model in Fig. 2 is that the amino group of the amino acid is very close to the amino group of the puromycin acting as the acceptor of the peptide; and even though the amino groups are only slightly charged at physiological pHs, it is likely that two amino groups close together would repel each other, thereby preventing reaction. This would explain the puzzling, but often-made observation that puromycin does not react with aminoacyl-tRNAs under ordinary conditions, even in systems with synthetic messengers where peptide chains are started with free amino acids, and the peptidyl site must be occupied by an aminoacyl-tRNA (e.g. 13,14). The only way to obtain aminoacyl-puromycins seems to be to carry out the reaction in extremely high salt concentrations (15), which is in accordance with the present model, for these conditions would be expected to overcome the charge effect.

I am grateful to the National Institutes of Health (grant no. 5 R01 AM 12959) for their support.

REFERENCES

1. M. B. Yarmolinsky and G. L. de la Haba, Proc. Natl. Acad. Sci., U.S. 45, 1721 (1959).
2. D. W. Allen and P. C. Zamecnik, Biochim. Biophys. Acta 55, 865 (1962).
3. D. Nathans, Proc. Natl. Acad. Sci., U.S. 51, 585 (1964).
4. R. R. Traut and R. E. Monro, J. Mol. Biol. 10, 63 (1964).
5. R. E. Monro, Nature 223, 903 (1969).
6. M. Cannon, R. Krug, and W. Gilbert, J. Mol. Biol. 7, 360 (1963).
7. R. E. Monro, J. Mol. Biol. 26, 147 (1967).
8. Y. Kuriki, I. Fukuma, and A. Kaji, J. Biol. Chem. 244, 1365 (1969).
9. Y. Kuriki and A. Kaji, J. Mol. Biol. 25, 407 (1967).
10. M. Sundaralingam and S. K. Arora, Proc. Natl. Acad. Sci., U.S. 64, 1021 (1969).
11. D. Nathans and A. Neidle, Nature 197, 1076 (1963).

12. R. H. Symons, R. J. Harris, L. P. Clarke, J. F. Wheldrake, and W. H. Elliott, Biochim. Biophys. Acta 179, 248 (1969).
13. J. D. Smith, R. R. Traut, G. M. Blackburn, and R. E. Monro, J. Mol. Biol. 13, 617 (1965).
14. S. Pestka, Proc. Natl. Acad. Sci., U.S. 64, 709 (1969).
15. B. E. H. Maden and R. E. Monro, Eur. J. Biochem. 6, 309 (1968).