The mode of action of the antitumor drug bouvardin, an inhibitor of protein synthesis in eukaryotic cells

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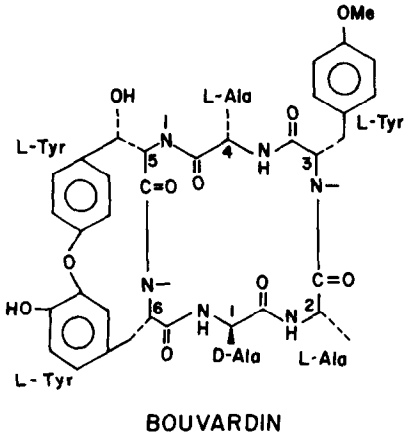
Bouvardain is an antitumor drug that inhibits protein synthesis in intact eukaryotic cells and cell-free systems. Our present studies have shown that bouvardin acts at the level of the 80 S ribosome in a site somehow involved with the interaction of EF1 and EF2. Indeed bouvardin inhibits EF1-dependent binding of aminoacyl-tRNA and EF2-dependent translocation of peptidyl-tRNA but does not affect the non-enzymic translocation since this reaction does not require EF2. The site of the 80 S ribosome involved in the interaction with bouvardin appears to be independent from the cycloheximide and the cryptopleurine binding sites since yeast mutants resistant to cycloheximide or cryptopleurine are sensitive to bouvardin.

Bouvardin *Protein synthesis* *Translocation* *Elongation* *Antitumor* *Antibiotic*

1. INTRODUCTION

Bouvardin is a cyclic hexapeptide (fig.1) which is isolated from *Bouvardia ternifolia* (Rubiaceae). This plant was used by ancient Mexican Indians as a general medicine and it is still used in Mexico as a drug against disentery and other diseases [1]. Bouvardin shows high antitumor activity in the P5 and B1 systems [1] as well as against P388 lymphocytic leukemia and B16 melanoma [2]. The effects of bouvardin on macromolecule synthesis by P388 tumor cell suspensions indicate that protein synthesis is inhibited preferentially to RNA and DNA synthesis [2]. These findings are interesting since other cyclic oligopeptides (gramicidins, tyrocidins, polymixins and octapeptins) are known to act in a completely different way; i.e., by affecting the structure of the cell membrane and producing lysis of bacterial and animal cells [3]. We have studied the mode of action of bouvardin on several steps of protein synthesis using resolved model systems from both yeast and reticulocytes. It has been concluded that bouvardin blocks polypeptide chain elongation by inhibiting both the GTP- and EF-2-dependent translocation of peptidyl-tRNA and the enzymic binding of aminoacyl-tRNA to 80 S ribosomes.



BOUVARDIN

Fig.1. Chemical structure of bouvardin.

2. MATERIALS AND METHODS

High salt washed ribosomes and polyribosomes from yeast, partially purified yeast supernatant fraction containing elongation factors EF-1 and EF-2, [^{3}H]Phe-tRNA, rabbit reticulocyte ribosomes and purified elongation factors EF-1 and EF-2 from reticulocytes were prepared as in [4,5]. The assay systems for poly(U)- and endogenous mRNA-directed polypeptide synthesis, enzymic binding of [^{3}H]Phe-tRNA to reticulocyte ribosomes, enzymic and non-enzymic translocation by yeast polyribosomes and the EF-2- and GTP-de-

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pendent translocation of *N*-Ac[^{3}H]Phe-tRNA were described in [4,6,7]. Bouvardin was a gift of Dr M. Chitnis. Radioactive compounds were obtained from Amersham International (Bucks).

3. RESULTS AND DISCUSSION

Bouvardin clearly inhibits protein synthesis in yeast cell-free systems. Thus, poly(U)-directed polyphenylalanine synthesis and endogenous mRNA-programmed polypeptide synthesis by these extracts were inhibited by 50% at 4×10^{-6} M and >90% at 4×10^{-4} M bouvardin (fig.2).

The inhibition of polypeptide chain elongation could be explained by the blocking of aminoacylation of tRNA. However, results obtained by ourselves (not shown) and others [2] indicate that the aminoacylation reaction takes place in the presence of bouvardin. Therefore, we have studied the effects of the cyclic hexapeptide on several model reactions of the elongation cycle. Neither peptidyl-[^{3}H]puromycin nor *N*-acetyl-[^{3}H]leucylpuromycin synthesis was affected by up to 4×10^{-3} M bouvardin as measured by the reaction of yeast polyribosomes with [^{3}H]puromycin or by the 'fragment reaction', respectively (not shown). It was concluded that the peptide bond formation step was not affected by bouvardin.

When the reaction of [^{3}H]puromycin with yeast polyribosomes was carried out in the presence of GTP and supernatant factors bouvardin did inhibit the formation of peptidyl-[^{3}H]puromycin (fig.3). Under these conditions translocation of peptidyl-tRNA takes place and consequently the inhibition of the reaction may be due to the action of bouvardin on translocation. The interaction of bouvardin with the translocation step is also sugested by the reversal of the inhibition of polyphenylalanine synthesis by increasing amounts of elongation factor EF-2 in a reticulocyte cell-free system (fig.4). However, bouvardin does not affect the non-en-

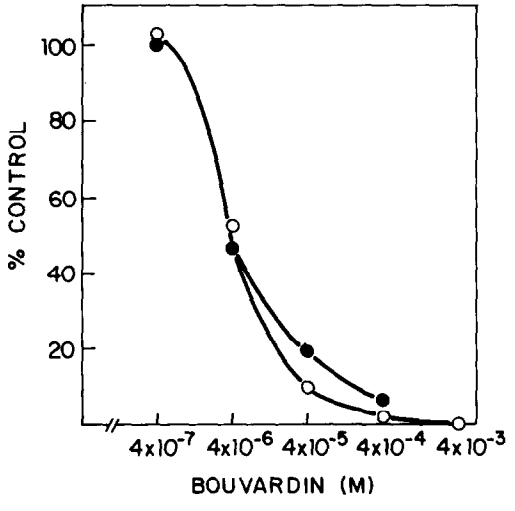

Fig.2. The effect of bouvardin on poly(U)-directed polyphenylalanine and endogenous mRNA-directed polypeptide synthesis by yeast ribosomal preparations. The reactions took place over 20 min. Other conditions were as in [4]. Incorporation in the controls in the absence of bouvardin was 180 and 15 pmol [^{3}H]phenylalanine for poly(U)- (○) or endogenous (●) mRNA-directed polypeptide synthesis.

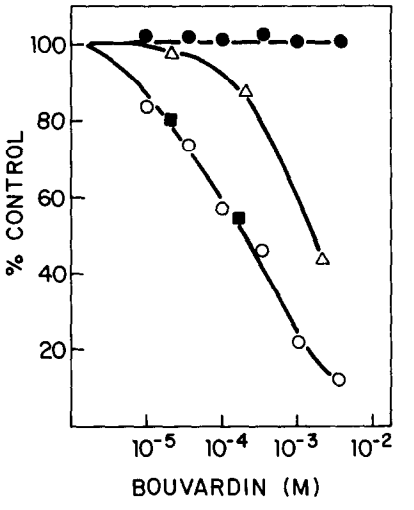

Fig.3. The effect of bouvardin on a number of model reactions of the elongation cycle of protein synthesis. Reactions were carried out as in Materials and Methods. (○) Enzymic translocation by yeast polyribosomes. 0.6 and 1.3 pmol peptidyl-[^{3}H]puromycin were synthesized in the controls in the absence and in the presence of added yeast supernatant factors, respectively. (■) Elongation factor EF-2- and GTP-dependent translocation of *N*-acetyl-[^{3}H]phenylalanine-tRNA by reticulocyte ribosomes; 1.1 pmol *N*-acetyl-[^{3}H]phenylalanyl-tRNA was translocated in the control in the absence of bouvardin. (●) Non-enzymic translocation by yeast polyribosomes; 11 pmol peptidyl-[^{3}H]puromycin were synthesized in the control in the absence of bouvardin. (△) Enzymic binding of [^{3}H]Phe-tRNA; 2.1 pmol [^{3}H]Phe-tRNA were bound in the absence of drug.

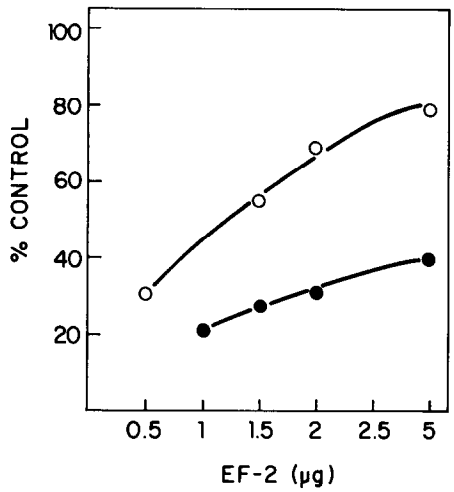

Fig.4. Effects of different amounts of elongation factor EF-2 on the inhibition of polyphenylalanine synthesis by bouvardin. Polyphenylalanine synthesis was performed as in [7]. Incorporation in the controls in the absence of drug was: 0.5, 2.1, 2.7 and 3.0 pmol [^{3}H]phenylalanine for 0.5, 1.5, 2 and 3 μg elongation factor EF-2 added. When required, bouvardin was present at: (○) 4×10^{-5} M or (●) 4×10^{-4} M.

zymic translocation [6] that takes place in the presence of high [K^+] and in the absence of elongation factor EF-2 and GTP (fig.2). An analogous situation has been found with several inhibitors of the enzymic translocation that bind to the small ribosomal subunit (cryptopleurine, emetine, tubulosine and hygromycin B) [8]. In contrast, cycloheximide and pederine that bind to the 60 S ribosomal subunit, block both the enzymic and non-enzymic reactions. Thus, bouvardin may act on the 40 S subunit, although there are no direct experimental bases for this suggestion.

The EF-1 and GTP-dependent binding of [^{3}H]phenylalanyl-tRNA to 80 S ribosomes was also inhibited by bouvardin, although to a lower extent than the inhibition of translocation (fig.2). This is the first instance that an antibiotic inhibits both aminoacyl-tRNA binding and translocation in a eukaryotic system. In prokaryotic systems, drugs of the thiostrepton group inhibited both reactions, evidence for the existence of overlapping binding sites for elongation factors G and Tu on the ribosome [9]. Analogously, our results with bouvardin also suggest that overlapping sites for EF-1 and EF-2 may exist in the eukaryotic ribosome. In support of this suggestion is the fact that aminoacyl-tRNA does not bind to GDP • 80 S ribosome • fusidic acid • EF-2 complexes [10].

Ribosomes from yeast strains resistant to cycloheximide or cryptopleurine are sensitive to bouvardin (unpublished). This result suggests that the interaction of bouvardin with the 80 S ribosome defines a new ribosomal binding site. The high content of tyrosine in bouvardin should allow one to label the molecule with ^{125}I. If the resulting derivatives were active, they could prove useful in the study of the binding parameters of bouvardin to the eukaryotic ribosome.

REFERENCES

[1] Jolad, S.J., Hoffmann, J.J., Torrance, S.J., Wiedhopf, R.M., Cole, J.R., Avora, S.K., Bates, R.B., Garginlo, R.L. and Kriek, G.R. (1977) J. Am. Chem. Soc. 99, 8040–8044.
[2] Chitnis, M., Alate, A. and Menon, R. (1981) Chemotherapy 27, 126–130.
[3] Gale, E., Cundiffe, E., Reynolds, P., Richmond, M. and Waring, M. (1981) in: The molecular bases of antibiotic action, Wiley, London, New York.
[4] Jiménez, A., Sánchez, L. and Vázquez, D. (1975) Biochim. Biophys. Acta 383, 427–434.
[5] Carrasco, L., Jiménez, A. and Vázquez, D. (1976) Eur. J. Biochem. 64, 1–6.
[6] Jiménez, A., Carrasco, L. and Vázquez, D. (1977) Biochemistry 16, 4727–4730.
[7] Carrasco, L., Barbacid, M. and Vázquez, D. (1973) Biochim. Biophys. Acta 312, 368–376.
[8] Dölz, H., Vázquez, D. and Jiménez, A. (1982) Biochemistry 21, 3181–3187.
[9] Cabrer, B., Vázquez, D. and Modolell, J. (1972) Proc. Natl. Acad. Sci. USA 69, 733–738.
[10] Carrasco, L. and Vázquez, D. (1973) FEBS Lett. 32, 152–156.