soluble enzymes; none of the hydrophobic or membrane-bound enzymes are assayable by our techniques. However, the systems surveyed include enzymes of intermediary metabolism (phosphoglucomutase, phosphoglucoisomerase, and triosephosphate isomerase) as well as nonspecific enzymes (esterase and acid phosphatase). It is interesting that the only purported isozyme unique to pollen, EST-A, is classified as nonspecific.

Of the 30 isozymes present in sporophytic stages, 18 were also found in pollen. If we count EST-A as unique to pollen, then 18 of the 19 pollen isozymes are also found in one or more of the sporophytic stages. All the pollen genes tested were expressed postmeiotically, apparently transcribed from genes in the haploid genome. The data suggest little divergence in the structural gene repertoire of sporophyte and gametophyte. By the same token, they support the concept that selection for genes expressed in the gametophytic stage could have a marked effect on the sporophytic generation, since many of these genes are expressed in both generations.

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13. We thank S. K. Jain for reviewing the manuscript and D. G. Hunt for editing. Supported in part by NSF grant DEB80-05542.

10 November 1980; revised 3 March 1981

Nalidixic Acid, Oxolinic Acid, and Novobiocin Inhibit Yeast Glycyl- and Leucyl-Transfer RNA Synthetases

Abstract. *Nalidixic acid and novobiocin inhibit the aminoacylation and pyrophosphate exchange activities of glycyl- and leucyl-transfer RNA synthetases from bakers' yeast. Similar types of inhibition are observed for both enzymes, suggesting similar mechanisms. The potency of these inhibitors is comparable to that observed for their inhibition of in vivo DNA synthesis in eukaryotic cells.*

Nalidixic acid (NA), oxolinic acid (OA), and novobiocin (NB) are potent inhibitors of DNA replication in *Escherichia coli* through their action on DNA gyrase (*1*). Both NA and OA appear to inhibit the nicking-closing activity of DNA gyrase (*2, 3*), and at low concentrations inhibit DNA replication (*4*). However, NA inhibition of transcription has been observed at higher concentration, and there is evidence that in *E. coli* the target of NA in this inhibition of transcription is also DNA gyrase (*5*).

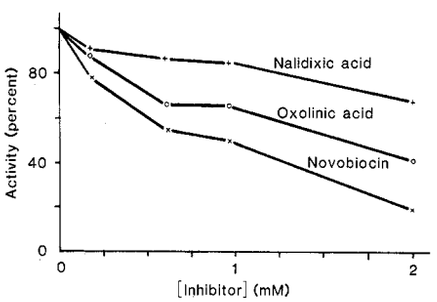

Fig. 1. Dependence of glycine synthetase activity on inhibitor concentration. Purified or partially purified glycyl-tRNA synthetase was mixed with different concentrations of drug. After equilibration at 30°C, a single mix of adenosine triphosphate (ATP), unfractionated tRNA, KCl, $MgCl_2$, and glycine in Hepes buffer at *p*H 7.2, 30°C, was added to the enzyme solution to give final concentrations of 1 m*M*, 2.4 μ*M*, 10 m*M*, 5 m*M*, 33.6 μ*M*, and 40 m*M*, respectively. Reaction was stopped with ice-cold 5 percent trichloroacetic acid after 3 minutes and samples were filtered through 0.45-μm Millipore filters, dried, and counted.

Low concentrations of NB block DNA replication, presumably by inhibiting the adenosinetriphosphatase activity of DNA gyrase in *E. coli* (*6*). Novobiocin also inhibits DNA replication in certain eukaryotic cells at much higher concentrations than those required for in vitro inhibition of prokaryotic DNA gyrase (*7, 8*). DNA polymerase α is also inhibited by NB in extracts of CV-1 monkey cells (*8*), but the target of NB which results in inhibition of eukaryotic DNA synthesis in vivo is not known.

We describe here our finding that NA, OA, and NB inhibit representatives of yet another class of enzymes, the aminoacyl-tRNA (transfer RNA) synthetases. Both yeast glycyl- (GST) and leucyl (LST)-tRNA synthetases are inhibited by these drugs, but at concentrations several orders of magnitude higher than those that inhibit *E. coli* DNA gyrase. Figure 1 shows a dose response curve for the three drugs in the aminoacylation catalyzed by GST. The yeast leucyl- and glycyl-tRNA synthetases used in our study were purified to near homogeneity (*9*).

We have determined the steady-state kinetic parameters for inhibition of the aminoacylation and pyrophosphate exchange reactions by these antibiotics with respect to the adenosine triphosphate (ATP) and tRNA substrates. Figure 2 shows the inhibition patterns for GST with respect to the ATP and tRNA substrates in the aminoacylation reaction. Similar data were obtained for the

Table 1. Summary of inhibition data for glycyl- and leucyl-tRNA synthetases.

Inhibitor	Substrate	Glycyl-tRNA synthetase		Leucyl-tRNA synthetase	
		K_m or K_I (m*M*)	Type*	K_m or K_I (m*M*)	Type*
	ATP	0.1		0.3	
	tRNA	0.6×10^{-3}		0.3×10^{-3}	
		Aminoacylation			
NA	ATP	0.8	C	2.7	C
NA	tRNA	1.3	?NC or C	2.0	NC
NB	ATP	0.9	NC	5.4	NC
NB	tRNA	0.2	UC	3.6	UC
		Pyrophosphate exchange			
	ATP	1.2		1.0	
NA	ATP	6.0	?NC or UC	3.9	NC
NB	ATP	3.5	NC	2.9	NC

*C, competitive; NC, noncompetitive; UC, uncompetitive.

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inhibition of the LST-catalyzed aminoacylation, and comparable kinetics of inhibition of the pyrophosphate exchange reaction catalyzed by both enzymes were observed (not shown).

In contrast to its effect on DNA gyrase, where it is a competitive inhibitor with respect to the ATP substrate (*1*), NB is a noncompetitive inhibitor of both synthetases, with respect to ATP, in the pyrophosphate exchange and aminoacylation reactions. Oxolinic acid is a better inhibitor of both DNA gyrase and GST than the closely related NA, although the difference is 20-fold in the case of the gyrase (*1*) and only two- to threefold for GST. While the potency of NA and NB as inhibitors of GST and LST is orders of magnitude less than for DNA gyrase, this range of inhibitor concentrations is comparable to that found inhibitory of in vivo protein synthesis in other eukaryotic cells (*8, 10*). Therefore, the aminoacyl-tRNA synthetases are candidates for targets of these drugs in the inhibition of protein synthesis in eukaryotic systems.

The inhibition of both glycyl- and leucyl-tRNA synthetases by NA and NB may be useful in understanding some aspects of the mechanism of this family of enzymes. The competitive inhibition of GST and LST by NA with respect to ATP (Fig. 2a) is unusual in that it is only manifested when the enzyme is incubated with the drug before the reaction is started. This requirement for prior incubation suggests that NA is acting to change the conformation or aggregation state of these enzymes from active to inactive ones. The observation of uncompetitive inhibition of NB with respect to tRNA in the LST-catalyzed reaction, taken with the ordered addition of substrates to this enzyme (*11*), is evidence that NB binds to a synthetase-tRNA complex. With the two possible exceptions noted in Table 1, NA and NB show identical inhibition patterns for GST and LST. These two synthetases differ structurally in that GST is oligomeric (*9, 12*), whereas LST is monomeric (*13–15*). Furthermore, the pyrophosphate exchange reaction of GST but not LST is stimulated by the tRNA substrate (*9, 12*). The similarity of their inhibition patterns is consistent with a common mechanism of action and common intermediates for these two superficially dissimilar aminoacyl-tRNA synthetases.

The structure of NB may provide an interesting clue about the nature of intermediates in the synthetase-catalyzed reactions. The crystal structure of NB (*16*) shows that it is sterically similar to a base pair, suggesting that a base pair may be an important locus of recognition between synthetase and tRNA substrate. In the case of LST, the uncompetitive inhibition and ordered addition of substrates is consistent with the existence of a ternary (or higher) complex of enzyme, tRNA, and NB. If this is so, a second unoccupied base-pair binding site may exist in the enzyme-tRNA complex, which is necessary for product formation.

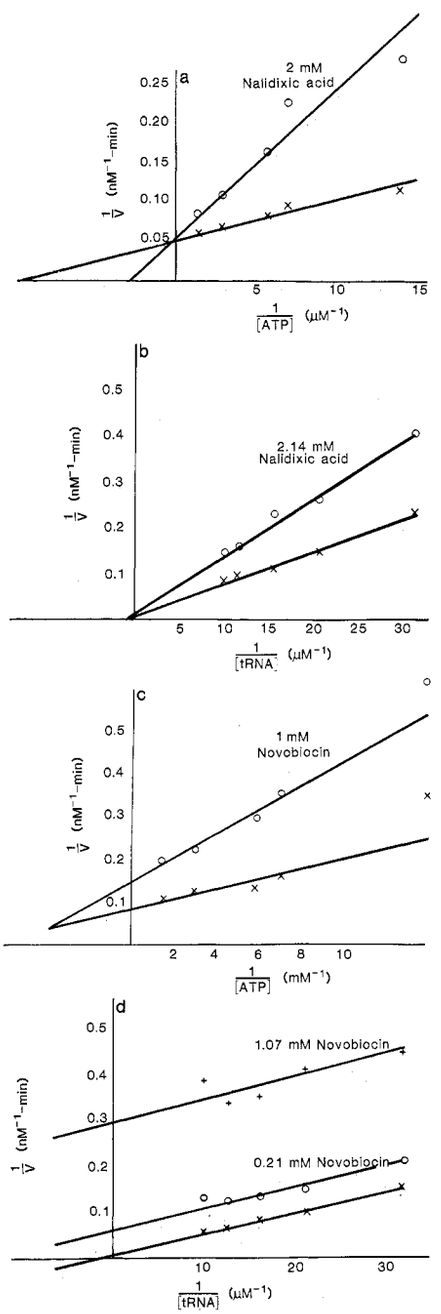

Fig. 2. Inhibition of glycyl-tRNA synthetase–catalyzed aminoacylation by nalidixic acid and novobiocin. Conditions and procedure are as in Fig. 1. The ATP concentrations varied from 0.1 m*M* to 1.0 m*M* and tRNA concentrations from 0.24 μ*M* to 2.4 μ*M* based on the extent of loading of unfractionated tRNA. (a) Inhibition by nalidixic acid with changing ATP concentration. (b) Inhibition by nalidixic acid with changing tRNA concentration. (c) Inhibition by novobiocin with changing ATP concentration. (d) Inhibition by novobiocin with changing tRNA concentration.

The fact that NA, OA, and NB are inhibitors of the reactions catalyzed by DNA topoisomerases and aminoacyl-tRNA synthetases does not in itself imply a relatedness of the mechanisms of these families of enzymes. However, these observations raise the possibility that the action of aminoacyl-tRNA synthetases on their tRNA substrates during the amino acid charging reaction has features in common with the transformations of closed circular DNA catalyzed by DNA gyrase. Further study of the different enzyme targets of these drugs may reveal unsuspected relationships among them, and may provide useful tools for understanding the mechanisms of enzymes which have macromolecular nucleic acid substrates.

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6 April 1981