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Folate Reductase and Specific Dihydrofolate Reductase Activities of the Amethopterin-Sensitive *Streptococcus faecium* var. *durans*

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Preliminary evidence is presented that indicates that the dihydrofolate reductase activity of amethopterin-sensitive *Streptococcus faecium* var. *durans* ATCC 8043 is separable into two dihydrofolate reductases, one of which also reduces folate.

In 1961, Blakley and McDougall isolated a dihydrofolate reductase highly specific for dihydrofolate (3) from the amethopterin-sensitive bacterium *Streptococcus faecalis* Rogers ATCC 8043. Later, we differentiated preparations of dihydrofolate reductase from amethopterin-sensitive *S. faecalis* (SF/O) and the amethopterin-resistant mutant *S. faecalis* A_k (SF/A_k) and noted that fractions eluted during molecular-sieve chromatography of SF/A_k extracts also catalyzed the reduction of folate more effectively than the comparable SF/O fractions (1). From another amethopterin-resistant mutant, *S. faecalis* A (SF/A or strain A), derived from SF/O by Hutchison (9), Hillcoat and Blakley obtained 100- to 500-fold purified preparations which also catalyzed the reduction of folate and dihydrofolate; evidence for more than one species of dihydrofolate reductase was presented (8). Nixon and Blakley isolated the two different dihydrofolate reductases of strain A as homogenous proteins in 1968 (10). One, designated "wild type," resembled the "single" reductase of strain 8043 in substrate specificity and in turnover. The other, designated "mutant type," differed in several properties and reduced folate and dihydrofolate. Recently, we reported (2) that SF/O, the parent strain of SF/A (strain A) and SF/A_k, produces at least two dihydrofolate reductases designated specific dihydrofolate reductase and folate reductase because the latter enzyme also reduces folate.

Since identification studies (4) established that the *Streptococcus* used in our laboratory is *S. faecium* var. *durans* (5, 6), and that SF/O and strain ATCC 8043 are not identical, we investigated the possibility of low-level folate reductase in strain 8043. We found evidence that strain 8043 also produces multiple forms of dihydrofolate reductase. The major form apparently is the species highly purified by Blakley and his associates (3, 8, 10) and is probably identical with the specific dihydrofolate reductase of SF/O. Another protein (or proteins), synthesized as an infinitesimally small percentage of the total protein, has folate reductase activity. Weakly detectable in cell extracts, "folate reductase" can be lost during purification of "dihydrofolate reductase" activity. The problematic recognition of folate reductase of strain 8043 is herein emphasized.

Bacterial cultivation, purification, and enzyme assay methods have been described previously (1, 2). One folate reductase unit was defined as the amount required to reduce 1 nmole of folate per min at 37 C, and one dihydrofolate reductase unit was defined as the amount required for the reduction of 1 nmole of dihydrofolate per min at 30 C under the standard conditions (2).

A stab-culture of strain 8043 in the medium of Flynn et al. (7) was used. Exponential cells growing in purine- and pyrimidineless medium with folate (10 μg/liter) were harvested and washed by resuspension in 10 mM potassium phosphate-1 mM ethylenediaminetetraacetic acid (pH 7.4; standard buffer); the cell extract was then prepared (2). Dihydrofolate reductase activity of this extract resembled that of SF/O (24 and 31 units/mg of protein, respectively). Since unidentified diazotizable material and other factors interfered with determination of folate reductase activity of extracts, small portions of extracts were assayed. Comparable assays estimated folate reductase activity of strain 8043 at

50% of SF/O activity (0.013 and 0.024 units/mg of protein, respectively).

The extract of strain 8043 was subsequently treated with protamine sulfate, and the ammonium sulfate fraction which was shown to contain 88 to 90% of the folate reductase activity of the SF/O extract (2) was prepared. Polyacrylamide gel (Bio-Rad, P-60) filtration (Fig. 1) resolved dihydrofolate reductase activity into two elution peaks. The larger peak (fractions 62 to 71) represented 90% of the dihydrofolate reductase activity of the extract. Peak fractions 65 to 67 showed 200- to 250-fold purification. The extremely small peak (fractions 46 to 55 which accounted for 2.6% of the dihydrofolate reductase activity of the extract) also reduced folate. Absence of diazotizable amine in acidified "folate reduction" mixtures which contained the potent reductase inhibitor amethopterin substantiated the enzymatic reduction of folate.

The apparent irregular elution of folate reductase is partially attributed to erratic elution of unidentified diazotizable material (slightly higher in fractions 40 to 50) which was presumably protein-bound. Since it contributed to absorbancy at 560 nm (2), enzyme activity values of fractions were corrected accordingly. Thus, the jagged elution pattern of folate reductase has perhaps meager significance. Elution of folate reductase-like activity with the exclusion front is probably significant. Whether the excluded material with folate reductase activity reflects a specific folate reductase which cannot reduce dihydrofolate or a nonspecific reduction remains unanswered.

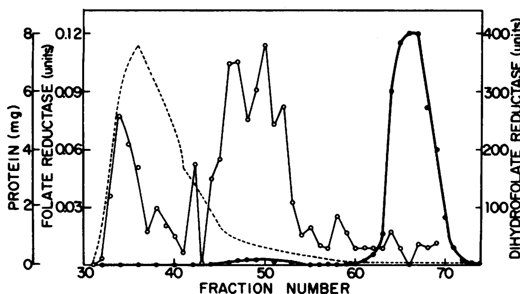

FIG. 1. *Polyacrylamide gel filtration behavior of total protein (broken line), folate reductase (○), and dihydrofolate reductase (●) activities of an ammonium sulfate fraction (0.3 to 0.7 saturation) of an extract of S. faecium var. durans ATCC 8043; a column of Bio-Gel P-60 (2.5 by 92.5 cm) was used at 4 C. Protein and enzyme activity values of the effluent fractions (3.5 ml) were computed after assay on the basis of 100 mg of total protein of the cell extract.*

Synthesis of almost subliminal folate reductase activity and an abundant and stable specific dihydrofolate reductase by strain 8043 under certain growth conditions could have contributed to the finding of a single dihydrofolate reductase (3, 8, 10). Accordingly, a single folate reductase in strain 8043, as concluded from studies with an amethopterin-resistant mutant, is improbable (11).

In emphasizing an earlier recommendation, "absolute description and definition of organism and medium" and "strain variation" among the amethopterin-sensitive strains of *S. faecium* var. *durans* (4), we urge the differentiation of amethopterin-resistant mutant strains of nonidentical parent strains. Caution in generalizations and speculative conclusions may also prevent seemingly conflicting findings.

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