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The gel-filtration behaviour of dihydrofolate reductases from culture forms of trypanosomatids

Inhibitors of dihydrofolate reductase (5,6,7,8-tetrahydrofolate:$NADP^+$ oxidoreductase, EC 1.5.1.3) such as pyrimethamine and trimethoprim are useful agents in the chemotherapy of malaria. It is particularly interesting therefore that FERONE *et al.*[1] have reported recently that the molecular weight, estimated by behaviour on Sephadex gel, of the dihydrofolate reductase of the rodent malaria parasite, *Plasmodium berghei*, is 190 000, a value about 10-fold higher than those determined for dihydrofolate reductases from mammalian[2,3] as well as avian[4], nematode[5] and bacterial sources[6]. We have recently examined the properties of dihydrofolate reductases from a number of trypanosomes[7] and related trypanosomatid flagellates[8]. We have now estimated by the Sephadex gel-filtration method that the molecular weights of the enzymes in three of these protozoa, *Trypanosoma (Trypanozoon) rhodesiense*, *Trypanosoma (Schizotrypanum) cruzi* and *Crithidia oncopelti* fall in the range 100 000–200 000, suggesting that a disproportionately high molecular weight is not a unique feature of the plasmodial enzyme but may be characteristic of dihydrofolate reductases of protozoal origin.

Blood forms of *T. (T.) rhodesiense* and *T. (S.) cruzi* were not available in sufficient quantities for these studies. Since no differences have so far been reported between the dihydrofolate reductases of the blood and culture forms of these two species[7], culture forms grown for us at the Microbiological Research Establishment, Porton, Wilts, were used. *T. (T.) rhodesiense* was grown in a modification of Pittam's medium[9] and *T. cruzi* (Strain Y) in a medium described by GUTTERIDGE *et al.*[10]. *C. oncopelti* was grown at Mill Hill in the peptone-containing medium first used for the organism by NEWTON AND HORNE[11]. After a thorough washing, all preparations were acetone-powdered and stored at −20°. To check the validity of our procedures, we also prepared acetone powders of chicken liver, since the molecular weight of its dihydrofolate reductase (23 000) had already been determined by others[4]. Similar preparations of rat liver served as a source of mammalian reductase. The molecular weight of this enzyme had not been reported but that of other mammalian reductases have been found by others[2,3] to be around 20 000. An extract of each powder was prepared as described previously[7] and the behaviour of its dihydrofolate reductase activity on a column of Sephadex G-200 was investigated in the manner described by ANDREWS[12].

The dihydrofolate reductase activity of extracts of *T. (T.) rhodesiense* appeared in two peaks, one having a V_e/V_0 ratio of 1.55 and the other 1.73. By means of ANDREWS'[12] plot of V_e/V_0 ratios, we estimated that these data indicated apparent molecular weights of 190 000 ± 10% and 135 000 ± 10%, respectively. The designation of ± 10% indicated that if the peaks were shifted by one fraction (3.5 ml) in either direction, the molecular weights would vary by that amount. The dihydrofolate reductase activity of extracts of *C. oncopelti* also appeared as two peaks, with V_e/V_0 ratios of 1.50 and 1.79, indicating apparent molecular weights of 200 000 and 100 000, respectively. In contrast, the activity of extracts of *T. (S.) cruzi* was not resolved in two major components; rather, we observed a band of activity with a peak V_e/V_0

ratio indicating a molecular weight of about 100 000. Dihydrofolate reductase activity in crude extracts of chicken liver acetone powders appeared in two peaks, one having a V_e/V_0 ratio of 2.27 and the other, 2.45, indicating molecular weights of 38 000 and 25 000, respectively. The molecular weight of the latter form is in agreement with that (23 000) reported by KAUFMAN AND GARDINER[4]. It is possible that the heavier "isozyme" is a dimer; multiple forms of dihydrofolate reductase have been described recently in preparations obtained from chicken liver[13] and from bacterial sources[14,15]. Dihydrofolate reductase activity in crude extracts of rat liver acetone powders appeared in a single peak having a V_e/V_0 ratio of 2.41; the molecular weight of the rat liver reductase was estimated to be approx. 27 000, a value reasonably close to those reported for other reductases of mammalian origin—20 000 (ref. 2) and 19 000–23 000 (ref. 3).

Dihydrofolate reductases from different sources exhibit differential sensitivity to inhibition by derivatives of 2,4-diaminopyrimidine and related heterocycles, particularly trimethoprim (2,4-diamino-5-(3′,4′,5′-(trimethoxybenzyl) pyrimidine) and B.W. 57-43 (1-(*p*-butylphenyl)-1,2-dihydro-2,2-dimethyl-4,6-diamino-*s*-triazine)[5,7,8,16–19]. The drug sensitivities of all the partially purified dihydrofolate reductases did not differ significantly from those of the enzymes from the corresponding crude extracts (Table I). Furthermore, the drug sensitivities of chromatographically distinct forms of either the trypanosomal or the crithidial reductases were essentially the same.

In summary our data indicate that the molecular weights of the dihydrofolate reductases of the three protozoal flagellates examined are in the range 100 000–200 000 (Table I). Only the molecular weight of dihydrofolate reductase of another protozoan

TABLE I

COMPARATIVE SENSITIVITY OF DIHYDROFOLATE REDUCTASES TO INHIBITION BY TRIMETHOPRIM AND B.W. 57-43

Source of enzyme	*Estimated mol. wt.*	*Concn. of drug (μM) for 50% inhibition*	
		Trimethoprim	*B.W. 57-43*
T. (T.) rhodesiense (culture)			
Eluate I	190 000	1.20	12
Eluate II	135 000	0.75	12
Literature value[7]		0.75	7
T. (S.) cruzi (culture)			
Eluate	100 000	20	8
Literature value[7]		10	5
C. oncopelti (culture)			
Eluate I	200 000	250	66
Eluate II	100 000	250	100
Literature value[8]		250	66
Rat liver			
Eluate	27 000	310	0.20
Literature value[17]		260	0.14
Chicken liver			
Eluate I	38 000	—	—
Eluate II	23 000	320	0.23
Literature value[19]		350	0.30

(P. berghei) and a mutant strain of *Diplococcus pneumoniae*[20] have been reported, thus far, to be in this range. The possibility that our estimates of molecular weight are too high due to extractive or separatory artefacts appears unlikely since, by means of similar preparatory and analytical methods, we have been able to confirm previous observations concerning the relatively low molecular weight of chicken liver dihydrofolate reductase. Whether dihydrofolate reductases with high molecular weights are characteristic of protozoa is a question that must be answered by further comparative biochemical studies.

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