THE ASSOCIATION-DISSOCIATION OF FORMYLTETRAHYDROFOLATE SYNTHETASE AND ITS RELATION TO MONOVALENT CATION ACTIVATION OF CATALYTIC ACTIVITY

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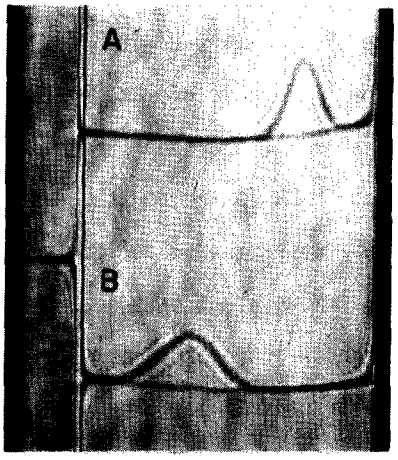
Formyltetrahydrofolate synthetase (E.C. 6.3.4.3) has been shown to require monovalent cations for enzyme activity (Whitely & Huennekens, 1962). Similar observations were reported concerning this requirement for the activity of the crystalline enzyme isolated from *Clostridium cylindrosporum* (Himes & Wilder, 1965). The latter authors showed that this enzyme was fully active in the presence of potassium, ammonium, and rubidium ions, partly active in the presence of cesium ions, and essentially inactive in the presence of sodium, lithium, or Tris ions. They suggested, on the basis of kinetic data, that a conformational change of the protein occurs in the absence of an activating cation that partially or completely destroys the binding sites of two of the substrates of the reaction, tetrahydrofolate and formate.

We would like to present evidence here that the enzyme dissociates into four subunits in the absence of potassium, ammonium, or rubidium ions, and to suggest that, although the dissociation of the enzyme into subunits could only be demonstrated, for technical reasons, at protein concentrations much higher than those used to determine catalytic activity, this behavior provides an alternate explanation for the observed requirement of monovalent cations for catalytic activity. The dissociation occurs under unusually mild conditions--the removal of the specific cations K^+, NH_4^+, or Rb^+ from a medium 50 mM with respect to Tris·HCl. A close correlation exists between the ions required for maintaining the

enzyme in the associated form and those required for catalytic activity. The dissociated form of the enzyme can be reassociated by the addition of K^+, and the catalytic activity is also restored by this treatment.

Experimental--The purification of formyltetrahydrofolate synthetase from *C. cylindrosporum*, the assay of its activity, and the materials used were essentially those previously described (Himes & Rabinowitz, 1962a). The effect of various monovalent cations was determined under assay conditions described by Himes & Wilder (1965). Monovalent cations were used in the form of their chloride salts. Ultracentrifuge patterns were determined with the Spinco model E analytical ultracentrifuge. The molecular weights were determined according to the method of Yphantis (1964) and the data was processed on the IBM computer with a program formulated by Dr. R. D. Dyson (personal communication).

Results--The enzyme migrates in a centrifugal field as a single component in the presence of 50 mM K^+, NH_4^+, or Rb^+. The molecular weight of the enzyme calculated from high speed equilibrium determinations under these conditions is about 230,000, in agreement with the results previously reported by Himes & Rabinowitz (1962b). Under the same conditions, but in the presence of 50 mM Na^+, Li^+, Tris, Cs^+, or Mg^{++}, the molecular weight of the enzyme is 58,000. These results suggest that formyltetrahydrofolate synthetase may exist as a monomer or a tetramer depending on the nature of the monovalent cation present. A graphic representation of the sedimentation velocity patterns is shown in Fig. 1 in 50 mM KCl (A) and in 50 mM NaCl (B). Schlieren patterns similar to those obtained in KCl were also obtained when this salt was replaced with NH_4Cl or RbCl, and schlieren patterns similar to those obtained in NaCl were obtained when this salt was replaced with LiCl, CsCl, $MgCl_2$, or Tris·HCl. The amount of monomer or tetramer found depends on the monovalent ion present and its concentration. The per cent of monomer or tetramer under a variety of conditions is shown in Table I. The enzyme dissociates



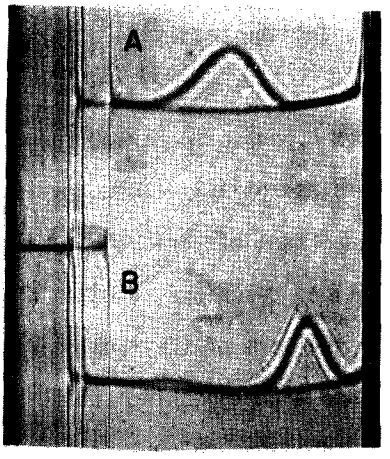


Fig. 1 Fig. 2

Fig. 1. Dissociation of formyltetrahydrofolate synthetase. After equilibration of the enzyme against the buffer solution, sedimentation velocity determinations were done at 22° in a 30 mm double sector cell at a protein concentration of about 2 mg/ml. Schlieren patterns were obtained 45 minutes after attaining a speed of 50,740 rpm at an angle of 60° for the schlieren diaphram. The positive cell (A) contained 50 mM KCl, and the negative cell (B) contained 50 mM NaCl. The direction of sedimentation is left to right.

Fig. 2. Reassociation of formyltetrahydrofolate synthetase. The positive cell (A) contained the enzyme in 0.05 M Tris·HCl, pH 7.9, 0.05 M 2-mercaptoethanol. The negative cell (B) contained enzyme from the same preparation, but KCl was subsequently added to make the final concentration 50 mM. The conditions used for the centrifugation and for obtaining the schlieren patterns were the same as those described in Fig. 1.

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completely in the presence of 50 mM Cs^+, Na^+, Tris, or Mg^{++}. Although it exists mainly in the form of the monomer in the presence of 50 mM Li^+, some tetramer could also be detected. It exists completely associated in the presence of 50 mM K^+, NH_4^+, or Rb^+. However, reduction of the K^+ concentration to 5 mM results in complete dissociation of the enzyme.

The catalytic activity of each preparation was also determined, with the results shown in Table I. As previously reported by Himes &

Table I. The Effect of Cations on the Catalytic and Physical Properties of Formyltetrahydrofolate Synthetase.

Salt Concentration	Catalytic Activity		Physical State	
			Tetramer	Monomer
mM	*units/mg*	%	%	%
KCl, 50	37,600	100	100	0
10	13,200	35	29	71
5	8,115	22	0	100
NH_4Cl, 50	37,200	99	100	0
RbCl, 50	38,113	101	100	0
CsCl, 50	13,868	37	0	100
10	6,156	16	0	100
NaCl,500	8,300	22	0	100
50	4,600	12	0	100
LiCl, 50	3,800	10	6	94
Tris·HCl, 70	3,112	8	0	100
50	1,661	4	0	100
10	2,101	6	0	100
$MgCl_2$, 50	1,361	4	0	100
10	1,158	3	0	100
5	3,560	9	0	100

The purified enzyme suspended in 50% ammonium sulfate, 0.02 M Tris, pH 7.5, 0.05 M 2-mercaptoethanol, was dialyzed for a total of 20 hours against four changes of 700 volumes each of the 0.02 M Tris·HCl, pH 7.5, 0.05 M 2-mercaptoethanol containing the salt concentration shown in the table. Schlieren patterns of the dialyzed enzymes were obtained under the conditions described in Fig. 1 and the per cent of the enzyme in the monomeric and tetrameric form was calculated from these patterns. The specific activities shown in the table were obtained under the assay conditions described by Himes & Wilder (1965) in which the catalytic activity was determined in a reaction mixture containing the salt concentration indicated.

Wilder (1965), the enzyme shows maximal activity in the presence of 50 mM K^+, NH_4^+, or Rb^+. The activity is drastically reduced in the same concentration of Na^+, Li^+, or Tris, or in 5 mM K^+. In control experiments, the addition of 50 mM K^+ to the inactive preparations restored full enzymic activity.

The dissociation observed in the absence of an activating cation (Fig. 2A) could be reversed by the addition of K^+ (Fig. 2B). The reversibility, under the conditions tested, is not altogether complete. The reassociated enzyme has almost full enzymic activity.

Discussion--Since the relationship established between the various cations and their ability to dissociate or associate the enzyme was done at protein concentrations approximately 200,000 times greater than those used to measure catalytic activity, it is possible that under the conditions for the assay of activity, the ratios of monomer to tetramer established by centrifugal analysis do not apply. However, the correlation between the catalytic activity and the physical state of the enzyme strongly suggests that the reason for the inactivity of the enzyme in the absence of certain cations is that the enzyme is dissociated into subunits that are catalytically inactive.

From the kinetic data of Himes & Wilder (1965), this inactivity of the enzyme may result from the disappearance of the tetrahydrofolate or formate binding sites either by virtue of the fact that the intact sites do not exist on the subunit, or that the active site is formed from portions of each subunit when they associate.

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REFERENCES

Himes, R. H., and Rabinowitz, J. C., *J. Biol. Chem.*, 237, 2898 (1962a).

Himes, R. H., and Rabinowitz, J. C., *J. Biol. Chem.*, 237, 2903 (1962b).

Himes, R. H., and Wilder, T., *Biochim. Biophys. Acta*, 99, 464 (1965).

Whitely, H. R., and Huennekens, F. M., *J. Biol. Chem.*, 237, 1290 (1962).

Yphantis, D. A., *Biochemistry*, 3, 297 (1964).