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A new cofactor in the conversion of serine to glycine

Cell-free extracts of an unidentified *Clostridium*, strain HF[1], catalyze the net production of glycine from serine. The fate of the β-carbon of serine has not yet been determined. Upon extensive dialysis of the extracts, glycine production shows a clear dependence on DPN, Mn^{++} and pyridoxal phosphate (see Experiment I). Following treatment with Dowex 1 HCl and subsequent overnight dialysis, the system can no longer be reactivated by these three cofactors. A fourth factor obtained from boiled extracts of *Clostridium cylindrosporum* is required in addition; it shall be referred to as Co C. Experiment II demonstrates that Co C is essential, and that its presence does not abolish the requirements for DPN, Mn^{++} and pyridoxal phosphate.

Co C is not identical with any of the folic acid derivatives found active in the systems of SAKAMI[2] and BLAKLEY[3]. Experiment II demonstrates that in this system folic acid and anhydroleucoverin are inert, but that citrovorum factor (C F) and tetrahydrofolic acid (*thf*) have an effect. In contrast to Co C, which is active at catalytic levels*, good activity is obtained with C F and *thf* only at substrate concentrations. Furthermore, DPN is not required when these compounds are used, (inhibition by DPN is usually observed) whereas DPN is required for activity with Co C. Another basis for distinguishing Co C from C F and *thf* is that saturation curves reveal that C F can never give as good activity as Co C, and that *thf* can at substrate concentrations give better activity (perhaps as a stoichiometric acceptor for the one carbon piece). Co C is not attacked by potato nucleotide pyrophosphatase. It is absorbed at low pH on Norite, and can be removed by ammoniacal ETOH; it is stable to boiling in dilute alkali (0 05 *N*) and is destroyed under similar conditions in dilute acid. Co C can be distinguished chromatographically from folic acid, C F, anhydroleucoverin, and *thf*; it has repeatedly been recovered from a fluorescent spot on paper chromatograms in various solvent systems.

Experiment I

The assay mixture contains the following substances in a total volume of 0.6 ml: 20.0 μM DL-serine**, 0.02 μM DPN, 2.0 μM $MnSO_4$, 0.02 μM pyridoxal phosphate, 0.04 ml *M* K-phosphate buffer pH 6.5, and 0.2 ml of an enzyme preparation (20 mg protein/ml) which was dialyzed 43 hours against 0.01 *M* phosphate buffer pH 7.2. The samples were incubated 2 hours at 28° C. The control value in the absence of serine (= 0.046 μM) is subtracted from each experimental value given.

Sample	*Omitted from assay mix*	*μM glycine formed*†
1	—	0.314
2	DPN	0.078
3	Mn^{++}	0.108
4	pyridoxal phosphate	0.134

* The most highly purified preparation of Co C obtained thus far by charcoal and paper chromatography has an absorption peak at 275–280 mμ. Assuming that the molecular extinction coefficient is the same as that for the folic acid derivatives ($\varepsilon_{280(max)}$ = 18,000–26,000) UV absorption at 280 mμ indicates that Co C is active at less than 0.01 the concentration of the glycine formed.

Experiment II

The assay mixture contains the following substances in a total volume of 0.4 ml: 6.0 μM DL-serine**, 0.02 μM DPN, 2.0 μM $MnSO_4$, 0.02 μM pyridoxal phosphate, 0.04 ml M K-phosphate buffer pH 6.5, and 6 units of enzyme. One enzyme unit yields 0.1 μM glycine at saturation levels of Co C after two hours' incubation at 38° C. A unit of Co C is an amount (< 0.001 μM)[2] yielding 0.1 μM glycine in the presence of one unit enzyme. The control value in the absence of serine ($= 0.012$ μM) is subtracted from each experimental value given.

Sample	*Omitted from assay mix*	*Special addition*	*μM glycine formed*†
1	—	—	0.016
2	—	*ca.* 6.0 units Co C	0.600
3	DPN	*ca.* 6.0 units Co C	0.202
4	Mn^{++}	*ca.* 6.0 units Co C	0.268††
5	pyridoxal phosphate	*ca.* 6.0 units Co C	0.409
6	—	0.02 μM folic	0.032
7	—	0.05 μM anhydroleucoverin	0.004
8	—	0.006 μM C F	0.080
9	—	0.02 μM C F	0.180
10	—	0.10 μM C F	0.280
11	DPN	0.10 μM C F	0.324
12	Mn^{++}	0.10 μM C F	0.088
13	pyridoxal phosphate	0.10 μM C F	0.312
14	—	0.006 μM *thf*	0.068
15	—	0.06 μM *thf*	0.120
16	—	0.60 μM *thf*	0.350
17	DPN	0.60 μM *thf*	0.412
18	Mn^{++}	0.60 μM *thf*	0.066
19	pyridoxal phosphate	0.60 μM *thf*	0.288

** For dialyzed preparations, the optimum serine concentration is 20.0 μM, as opposed to 6.0 μM for Dowex-treated enzyme. This difference is probably due to fewer side reactions involving serine in the presence of Dowex-treated enzyme.

† The glycine is assayed by chemical methods[4].

†† Some Mg^{++} was added with the Co C due to the method of purification. Since Mg^{++} can replace the Mn^{++} requirement to some extent, this value is unusually high.

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