The enzymic equilibration of L-serine with O-phospho-L-serine

Partially purified enzyme preparations from rat liver have been found to catalyse the following reactions:*

1. P-L-serine → L-serine + P_i
2. P-D-serine → D-serine + P_i
3. P-L-serine + 3-^{14}C-L-serine ⇌ 3-^{14}C-P-L-serine + L-serine

These reactions have also been observed in the independent studies of NEUHAUS AND BYRNE, as reported in an accompanying communication[1].

The Michaelis constant for P-L-serine in reaction 1 is quite low (approximately 10^{-5} M) while for P-D-serine in reaction 2 it is considerably higher (approximately $3 \cdot 10^{-3}$ M). There is an absolute requirement for Mg^{++} or other divalent cations for all three reactions.

The hydrolysis of both P-L-serine and P-D-serine is severely inhibited by L-serine, while comparable amounts of D-serine are without effect, as shown in Table I.

TABLE I

ENZYMIC HYDROLYSIS OF O-PHOSPHO-L-SERINE AND O-PHOSPHO-D-SERINE

Substrates added	*P_i released (μmole)*
1. 3 μmoles P-L-serine	0.32
2. 3 μmoles P-L-serine + 1 μmole L-serine	0.14
3. 3 μmoles P-L-serine + 1 μmole D-serine	0.32
4. 3 μmoles P-D-serine	0.45
5. 3 μmoles P-D-serine + 1 μmole L-serine	0.15
6. 3 μmoles P-D-serine + 1 μmole D-serine	0.46

Each tube contained 16 μmoles $MgCl_2$, 20 μmoles tris(hydroxymethyl)aminomethane, pH 7.5 and 0.8 mg of rat-liver enzyme purified by absorption on calcium phosphate gel and elution with 0.5 M ammonium sulfate. The final volume was 1.0 ml and the incubation was for 1 h at 37°.

TABLE II

THE ENZYMIC EQUILIBRATION OF L-SERINE WITH P-L-SERINE

Substrates added	*Percentage of radioactivity converted to P-serine*
Expt. 1	
1. 5 μmoles P-L-serine + 1 μmole 3-^{14}C-L-serine	31.5
2. 5 μmoles P-L-serine + 1 μmole 3-^{14}C-D-serine	1.7
Expt. 2	
1. 2 μmoles P-L-serine + 1 μmole 3-^{14}C-DL-serine	19.1
2. 2 μmoles P-D-serine + 1 μmole 3-^{14}C-DL-serine	0.61

The conditions of incubation were as described in Table I. At the end of the experiment, each tube was boiled, the protein removed by centrifugation, and the supernatant solution concentrated under a jet of air. The P-serine fraction was then separated from free serine by chromatography on paper, using 70% ethanol containing 0.02 M citrate buffer, pH 4.8, as solvent. The spots containing the P-serine and serine were cut out and counted in a gas-flow counter. The specific activity of the labelled serine was 135,000 counts/μmole in Expt. 1, and 131,000 counts/μmole in Expt. 2.

Enzyme preparations which catalyze reactions 1 and 2 also catalyze the enzymic equilibration of L-serine with P-L-serine, which may be studied by incubation of labelled L-serine with unlabelled P-L-serine, followed by chromatographic separation and counting of the P-serine fraction (Table II). However, only L-serine and P-L-serine participate in reaction 3; labelled D-serine is not converted to phosphoserine in the presence of unlabelled P-L-serine (Table II, Expt. 1) nor is labelled L-serine converted to phosphoserine in the presence of unlabelled P-D-serine (Table II, Expt. 2). It is not known whether the very small equilibration observed with P-D-serine in this experiment may be caused by a trace of P-L-serine in the sample of P-D-serine.

P-L-serine and P-D-serine are apparently hydrolyzed at the same enzymic site, since P-L-serine effectively displaces ^{32}P-D-serine from the enzyme (Table III).

* P-L-serine = O-phospho-L-serine; P-D-serine = O-phospho-D-serine; P_i = inorganic orthophosphate.

TABLE III

EFFECT OF P-L-SERINE ON HYDROLYSIS OF ^{32}P-D-SERINE

Substrates added	*Radioactivity released as P_i (counts/min)*
1. 2 μmoles ^{32}P-D-serine	8565
2. 2 μmoles ^{32}P-D-serine + 2 μmoles P-L-serine	275

Conditions of incubation were the same as in Table I, except that the time of incubation was 20 min. The radioactivity released as P_i was determined by a method based on that of ERNSTER, ZETTERSTROM AND LINDBERG[3]. The specific activity of the ^{32}P-D-serine was 104,000 counts/min μmole.

A previous study of the enzymic hydrolysis of phosphoserine has been carried out by ICHIHARA AND GREENBERG[2] who did not observe the reactions described by NEUHAUS AND BYRNE[1] and by us.

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Department of Biochemistry, University of Chicago, Chicago, Ill. (U.S.A.)

LOUISE FENCIL BORKENHAGEN
EUGENE P. KENNEDY

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O-Phosphoserine phosphatase*

The following reactions have been found to be catalyzed by enzyme preparations from chicken and rat liver**.

$$\text{O-phosphoserine} \longrightarrow \text{serine} + \text{orthophosphate} \quad (1)$$

$$\text{O-phospho-L-serine} + {}^{14}C_3\text{-L-serine} \longrightarrow {}^{14}C_3\text{-O-phospho-L-serine} + \text{L-serine} \quad (2)$$

The phosphatase activity, reaction 1, was purified 20 fold starting with aqueous extracts of the acetone powder of chicken liver. The purified enzyme preparation dephosphorylates L-PS*** at the rate of 1.81 μmoles/10 min/mg protein at 38°. The final concentration of the components in the test system were 0.01 *M* $MgCl_2$; 0.05 *M* succinate buffer, pH 5.90; 0.01 *M* substrate and enzyme. After deproteinization, the orthophosphate formed was determined by the method of DRYER, TAMMES AND ROUTH[1]. With respect to reaction 1, the purified preparation is highly specific for PS. At low substrate concentrations it is specific for L-PS ($K_m = 5.8 \cdot 10^{-5}$ *M*) while at high substrate concentrations it will dephosphorylate D-PS ($K_m = 4.2 \cdot 10^{-3}$ *M*) which confirms the observation of BORKENHAGEN AND KENNEDY[2] that rat-liver preparations dephosphorylate D-PS as well as L-PS. Identical maximum velocities were observed for both isomers. The only other substrate dephosphorylated at any appreciable rate is *p*-nitrophenyl phosphate which is due to a contaminating, uncharacterized phosphatase. These results are in contrast to the conclusion of ICHIHARA AND GREENBERG[3] that the major pathway for the cleavage of PS was carried out by a non-specific phosphatase present in rat-liver extracts.

L-Serine was found to be a very effective inhibitor ($K_i = 5.9 \cdot 10^{-4}$ *M*) of the phosphatase activity. DL-Homoserine (0.05 *M*) and DL-threonine (0.05 *M*) were without effect. LINEWEAVER-BURK plots[4] showed that the L-serine inhibition is uncompetitive.

The exchange of $^{14}C_3$-L-serine with L-PS, reaction 2, occurs at a significant rate in chicken- and rat-liver homogenates. This exchange is illustrated for a chicken-liver homogenate by the rapid

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** Similar and independent results are being published simultaneously by BORKENHAGEN AND KENNEDY[2].

*** The following abbreviations are used: PS, O-phosphoserine; L-PS, O-phospho-L-serine; D-PS, O-phospho-D-serine; $^{14}C_3$-L-serine, uniformly labelled L-serine; S.A., specific activity (counts/min/μmole).