INTERCONVERSION OF MYO- AND SCYLLO-INOSITOL WITH SIMULTANEOUS FORMATION OF NEO-INOSITOL BY AN $NADP^+$ DEPENDENT EPIMERASE FROM BOVINE BRAIN

Paul P. Hipps, William H. Holland and William R. Sherman

Departments of Psychiatry and Biological Chemistry
Washington University School of Medicine
St. Louis, Missouri 63110

Received May 31,1977

SUMMARY

An epimerase has been partially purified from bovine brain which interconverts myo- and scyllo-inositol with simultaneous production of neo-inositol. The enzyme(s) occur in the same unbound DEAE-cellulose fraction as the previously described scyllo-inositol:$NADP^+$ oxidoreductase. The epimerase has a pH optimum of 9.5, requires dithiothreitol for activity and utilizes only $NADP^+$ as cofactor.

INTRODUCTION

Two biochemical pathways are known by which scyllo-inositol is formed: the epimerization of myo-inositol and the reduction of myo-inosose-2. These conversions have been demonstrated to occur in the intact rat (1), in leaves of Calycanthus occidentalis (2), in locust (3) and cockroach (4) fat body extracts and in Streptomyces griseus (5). At present two pathways are also known for the biological synthesis of neo-inositol: the cyclization of mannose-6-P by the same enzyme preparation which catalyzes the conversion of glucose-6-P to myo-inositol-1-P (6) and, the epimerization of myo-inositol, by an enzyme present in extracts of cockroach fat body (4). In this report we describe our finding that enzymatic activity exists in cell-free preparations of bovine brain which converts myo-inositol both to scyllo- and neo-inositol and scyllo-inositol to myo- and neo-inositols. Inosose is also formed in these reactions which take place in the same enzyme preparation which has previously (7) been shown to reduce myo-inosose-2 to scyllo-inositol. The two activities have different pH profiles.

ISSN 0006-291X

myo-Inositol neo-Inositol scyllo-Inositol myo-Inosose-2

2,4,6/3,5-Pentahydroxy-cyclohexanone

MATERIALS AND METHODS

Enzyme Preparation: Either fresh or frozen bovine brain tissue was used for the preparation. After being weighed, the tissue was homogenized in a Waring blender using a volume of pH 7.5, 50 mM Tris-HCl buffer containing 0.2 mM dithiothreitol equal to the tissue weight. The homogenate was centrifuged at 15,000 x g for 1 hour and the supernatant subjected to pressure dialysis using an Amicon ultrafiltration apparatus with a PM-10 membrane. The volume of the supernatant was reduced 3.4-fold and 50 ml, containing 1.9 gm total protein, was pumped onto a 4 x 13 cm column of Whatman DE-52 DEAE-cellulose. The column had been prepared in pH 7.5, 50 mM Tris-HCl buffer containing 0.2 mM dithiothreitol and was eluted with the same buffer.

Enzyme Assay: The enzyme preparation was incubated with 5 mM inositol in 50 mM Tris buffer, pH 9.5, containing 1.25 mM pyridine nucleotide cofactor, 0.2 mM dithiothreitol and 1.5 mM NaN_3. After incubation at 37° for 20 min in a total volume of 200 ul the reaction was stopped by heating in boiling water for 10 min. Protein was precipitated by adding 25 ul of 1 M $ZnSO_4$; 500 ul of H_2O was added and, following centrifugation, a 500 ul aliquot was lyophilized and Me_3Si ethers of the products were prepared by shaking for 24 hours with 150 ul of pyridine: N,O-bis(trimethylsilyl)trifluoroacetamide: trimethylchlorosilane (1:1:0.2, v/v). A dilution curve of Me_3Si myo-, scyllo- and neo-inositol and of myo-inosose-2 was used for quantitation. Samples were analyzed by selected ion monitoring using a Finnigan 3200 gas chromatograph-mass spectrometer interfaced with a computer system developed at this institution (8). Chromatographic conditions were: 1.8 m x 0.6 cm OD glass column packed with 1% SE-30 on 80/100 mesh Gas Chrom Q with a helium flow of 30 cm^3/min and a column temp. of 195°. The mass spectrometer was operated at 70 eV ionization potential with the filament under emission control and the analyzer at 100°. The mass spectral fragment ions used were m/e 432, 433, 507 and 612 (M^+) for the inositols and m/e 538 (M^+) for inososes. These ions are specific for the inositols (9) and for inososes (10,11) under the conditions described. The ratio of the abundances of these ions is characteristic and, along with chromatographic retention time, provides validation of the specificity of the analyses.

Protein was determined by absorbance at 260 and 280 nm (12).

The enzymic assay as described was linear for at least 45 min.

Reagents: myo-Inositol, myo-inosose-2, NAD^+, $NADP^+$, NADH, NADPH and dithiothreitol were commercial samples (Sigma, Calbiochem). neo-Inositol (13) and scyllo-inositol (14) were synthesized.

RESULTS

When the previously described preparation of scyllo-inositol:$NADP^+$ oxidoreductase (7) was carried out in buffer containing 0.2 mM dithiothreitol,

TABLE 1. SUBSTRATE AND PRODUCT SPECIFICITY OF INOSITOL EPIMERASE[1]

	Inositol Product: pmoles/min/mg protein		
Substrate:Cofactor	myo-	neo-	scyllo-
myo-Inositol:$NADP^+$	-	1210	14
myo-Inositol:NAD^+	-	trace[2]	trace[2]
scyllo-Inositol:$NADP^+$	54	17	-
myo-Inosose-2:NADPH[3]	26	-	42
myo-Inosose-2:NADH[3]	0.03	-	0.2

[1] The enzyme preparation used contained 16.7 mg protein/ml (A280/260). Standard incubation conditions as described in text were used except as noted.
[2] Incubated for 5 hours under standard conditions.
[3] Incubations with 0.74 mM myo-inosose-2 were carried out for 5 hours, with 1.25 mM cofactor in 50 mM, pH 7.5 Tris HCl. The reaction is linear for 6 hrs.

inositol epimerase activity was found in the unbound DEAE "wash" fraction along with the scyllo-inositol oxidoreductase. The results shown in Table 1 are from the most extensively studied of five preparations and represent a 20 to 25-fold purification relative to the homogenate supernatant. In some of the other preparations the specific activity was reduced, but in all cases readily detectable epimerase activity was present. In the absence of dithiothreitol there was a 100-fold reduction in specific activity.

The epimerase, in addition to interconverting myo- and scyllo-inositols, produces large amounts of neo-inositol (Table 1 and Figure 1). From the product ratios it appears that neo-inositol is formed from myo-inositol, therefore, it is the second epimerization product when scyllo-inositol is the substrate.

The epimerase activity is highly specific for $NADP^+$. In a 5 hour incubation of myo-inositol with NAD^+ only a trace of scyllo- and neo-inositol were formed.

Inososes were also identified among the enzyme reaction products by the presence of ions typical of their Me_3Si derivative (10) (Figure 1). It was

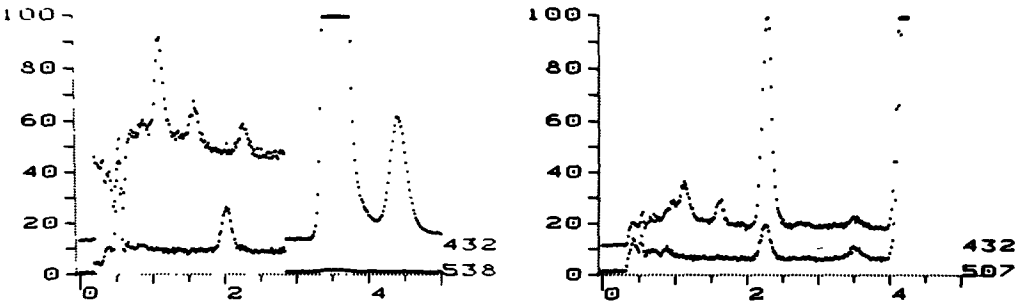

Figure 1 Selected ion monitoring traces from the gas chromatography-mass spectrometry of the Me_3Si derivatives of products from bovine brain inositol epimerase. Ordinate is percent intensity, abscissa is time, in minutes.
Left figure: scyllo-Inositol substrate. The ion m/e 432 is characteristic of Me_3Si inositols, m/e 538 is the molecular ion of Me_3Si inososes. The displacement of the traces at 2.8 minutes is to a lower level of amplification. The peak at 2.0 minutes (lower trace) is from inosose; at 2.3 minutes, neo-inositol; at 3.2-4 minutes, scyllo-inositol substrate; at 4.4 minutes, myo-inositol.
Right figure: myo-Inositol substrate. Both ions (m/e 432 and 507) are characteristic of Me_3Si inositols. The peak eluting at 2.3 minutes is neo-inositol, at 3.5 minutes, scyllo-inositol. The offscale peak at 4+ minutes is substrate myo-inositol.

not possible to quantitatively measure the amounts of the inososes which were present or to be certain of their origin[1], however, a minimum of 9 pmoles of myo-inosose-2 was measured in the experiment reported in Table 1 where myo-inositol was the substrate, and at least 90 pmoles when scyllo-inositol was the substrate.

The same enzyme preparation, when incubated with myo-inosose-2 at pH 7.5, used NADPH 200 times more effectively than NADH to form scyllo-inositol (Table 1). In previous studies with this preparation in the absence of dithiothreitol no NADH activity was found.

[1] Under the pH conditions of the incubation, myo-inosose-2 is rapidly enolized to more than twelve different inososes and enediols (11). During some analyses myochiro-inosose was present partially resolved chromatographically from myo-inosose-2 suggesting that such enolization had occurred. Therefore it is likely that four inososes are present, unresolved, in the elution envelope of myo-inosose-2. These are, in order of elution on SE-30: 2,3,5/4,6-pentahydroxy-cyclohexanone (myochiro-inosose); 2,3,4,5/6-pentahydroxycyclohexanone (myoepi-inosose); 2,4,6/3,5-pentahydroxycyclohexanone (scyllomyo-inosose, "myo-inosose-2") and 3,4,5/2,6-pentahydroxycyclohexanone (myoneo-inosose).

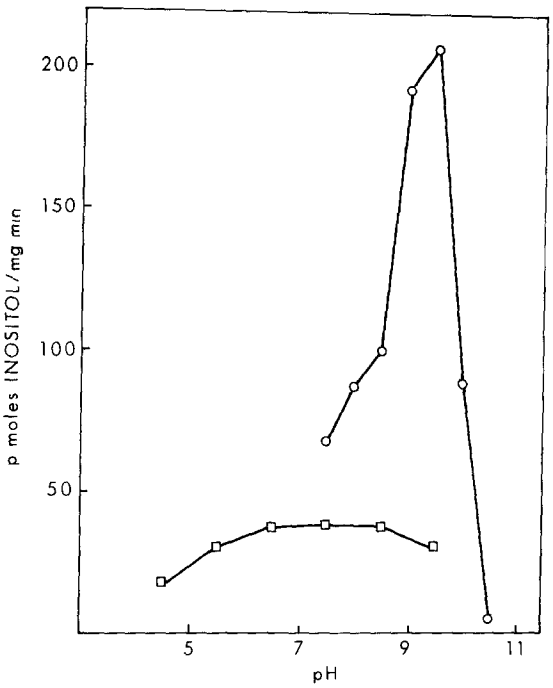

Figure 2 pH-Activity profiles of bovine brain epimerase (upper curve) and scyllo-inositol:NADP+ oxidoreductase (lower curve). The epimerase study used Tris-carbonate buffer, the oxidoreductase Tris-HCl. The epimerase curve was only tested for linearity at pH 9.5, the oxidoreductase at pH 4.5, 7.5 and 9.5. The oxidoreductase activity is multiplied by 100.

The pH-activity profile of the epimerase shows a sharp optimum at pH 9.5, with a 66% reduction in activity at pH 7.5 (Figure 2), in marked contrast with that of the scyllo-inositol oxidoreductase which has a broad pH-activity curve with an optimum near pH 7 .

DISCUSSION

The bovine brain epimerase resembles that found in extracts of cockroach fat body (3) in its ability to interconvert myo-, scyllo- and neo-inositol. Another similarity between the bovine and cockroach epimerases are their sharp alkaline pH optima and the presence, in both preparations, of an inosose reductase activity which has a broad, neutral pH optimum. Differences between the bovine brain and insect epimerases are; the requirement of the insect

enzymes (3,4) for NAD^+ rather than $NADP^+$, and, the dependence of the bovine enzyme on dithiothreitol for activity.

As with the insect studies, the interpretation of the results is complicated by the presence of two kinds of related enzyme activities and further complicated by the finding that both the epimerase and the reductase use the same dinucleotide cofactor. Thus the production of inososе(s) could be due either to: dissociation of the inososе from an epimerase, the presumption being that this is the epimerase intermediate; or the reversal of the scyllo-inositol:$NADP^+$ oxidoreductase. In the latter case, this reaction, while demonstrable, is difficult to detect at pH 7.5 in the absence of dithiothreitol (11).

Three arguments support the presence of two enzymes in the bovine brain preparations: 1) Two pH-activity profiles are apparent; 2) Two apparently distinct enzyme activities occur. While it is possible that the inososе reductase represents a half-reaction of the epimerase, most epimerases using an oxidation-reduction mechanism with a carbonyl-containing intermediate show tight binding of the reaction intermediates, i.e., neither reduced $NADP^+$ nor inososе would be expected to be released during the inositol epimerase reaction nor should either be accessible to the enzyme for a partial reaction. The formation of only 9 pmoles of inososе, during the reaction where 1210 pmoles of neo-inositol is formed from myo-inositol, seems compatible with a small amount of epimerase intermediate released during the reaction. However, the formation of 90 pmoles of inososе during the production of 54 pmoles of myo-inositol when scyllo-inositol is the substrate, in an enzyme preparation known to contain scyllo-inositol:$NADP^+$ oxidoreductase, seems best explained by reversal of the oxidoreductase; 3) The reductase activity tends to produce scyllo-inositol, the equatorial hydroxyl reduction product, whereas the epimerase reaction produces mainly axial hydroxyl reduction products (neo- and myo-inositol). Thus there appear to be two specificities which may represent two kinds of enzyme activity, however, it is clear that this question will be best answered by further study of the enzyme preparation.

ACKNOWLEDGEMENT

This work was supported by grants NS-05159, RR-00954 and AM-17904 from the National Institutes of Health.

REFERENCES

1. Posternak, J., Schopfer, W.H., Kaufmann-Boetsch, B. and Edwards, S. (1963) Helv. Chim. Acta 46, 2676-2685.
2. Scholda, R., Billek, G. and Hoffmann-Ostenhof, O. (1964) J. Physiol. Chem. 339, 23-32.
3. Candy, D.J. (1967) Biochem. J. 103, 666-671.
4. Hipps, P.P., Sehgal, R.K., Holland, W.H. and Sherman, W.R. (1973) Biochem. 12, 4705-4712.
5. Horner, W.H. and Thacker, I.H. (1968) Biochim. Biophys. Acta 165, 306-308.
6. Sherman, W.R., Goodwin, S.L. and Gunnell, K.D. (1971) Biochem. 10, 3491-3499.
7. Hipps, P.P., Eveland, M.R., Laird, M.H. and Sherman, W.R. (1976) Biochem. Biophys. Res. Comm. 68, 1133-1138; Hipps, P.P., Eveland, M.R. and Sherman, W.R. (1974) Federation Proceedings 33, 1560.
8. Berger, P.S., Norberg, P.D. and Holmes, W.F. (1975) Proceedings of the 23rd Annual Conference on Mass Spectrometry and Allied Topics, Houston, p. 507; Holmes, W.F., Norberg, P.D. and Scheifler, R.W. (1976) Proceedings of the 24th Annual Conference on Mass Spectrometry and Allied Topics, San Diego, p. 646-647.
9. Sherman, W.R., Eilers, N.C. and Goodwin, S.L. (1970) Org. Mass Spectrom. 3, 829-840.
10. Sherman, W.R., Stewart, M.A., Simpson, P.C. and Goodwin, S.L. (1968) Biochem. 7, 819-824.
11. Hipps, P.P. and Sherman, W.R., Unpublished Results.
12. Kalckar, H.M. (1947) J. Biol. Chem. 167, 461-475.
13. Angyal, S.J. and Matheson, N.K. (1955) J. Amer. Chem. Soc. 77, 4343-4346.
14. Stanacev, N.Z. and Kates, M. (1961) J. Org. Chem. 26, 912-918.