Journal of Neurochemistry, 1973, Vol. 20, pp. 889 to 891. Pergamon Press. Printed in Great Britain.

SHORT COMMUNICATION

Enzymatic desulphation of cerebroside-3-sulphate by chicken brain arylsulphatase A

(*Received* 17 *July* 1972. *Accepted* 26 *September* 1972)

IN EARLIER work from this laboratory it was shown that arylsulphatase of chicken brain resembles arylsulphatase A of other animal species in several of its properties but exhibits certain characteristics similar to that of arylsulphatase B (FAROOQUI and BACHHAWAT, 1971). Recently the arylsulphatase A of chicken brain was purified and it was demonstrated that the purified enzyme could desulphate cerebroside-3-sulphate also (FAROOQUI and BACHHAWAT, 1972). In the present report we have made a study of the kinetic properties of this unique arylsulphatase A purified from chicken brain using *p*-nitrocatechol sulphate and cerebroside-3-sulphate as substrates.

METHODS

In the assay of arylsulphatase A employing [^{35}S]cerebroside-3-sulphate as substrate, the reaction mixture contained 0·01 M-sodium acetate buffer pH 4·5, 70,000 c.p.m., [^{35}S]cerebroside-3-sulphate (22 nmol) and 30 μg enzyme protein (0·76 units) in a total volume of 0·1 ml. The tubes were incubated for 1 h at 37°C and the reaction was terminated by heating the tubes in a boiling water bath for 30 s. Immediately after heating the tubes were cooled in ice. The precipitated protein was removed by centrifugation. The separation of [^{35}S]sulphate and undegraded [^{35}S]cerebroside-3-sulphate was achieved by paper electrophoresis. A portion of the supernatant was streaked on Whatman 3 MM paper in the form of a 1 cm band. Paper electrophoresis was carried out in 0·03 M-sodium barbitone buffer pH 8·0 for 1·5 h at 250 V. The areas corresponding to [^{35}S]sulphate were cut out from the paper and the radioactivity was measured in a Packard Tri-Carb liquid scintillation counter as described by GEORGE and BACHHAWAT (1970). In the control reaction mixture the enzyme was added at the end of the incubation period. Using the assay technique it was observed that there was a linear relationship between the enzyme activity and the protein concentration.

In the assay of arylsulphatase A using nitrocatechol sulphate as substrate, the enzyme was assayed by incubation at pH 5·5 for 5 min as described by FAROOQUI and BACHHAWAT (1972).

[^{35}S]Cerebroside-3-sulphate was isolated from the brains of 17-day-old rats after intraperitoneal injection of carrier-free [^{35}S]sodium sulphate (20 μCi/g body wt.) as described earlier (FAROOQUI and BACHHAWAT, 1972). The specific radioactivity of the isolated cerebroside-3-sulphate was $3·5 \times 10^6$ c.p.m./mg. The solution of cerebroside-3-sulphate was prepared in Tween 20 (0·2% v/v) and used as substrate.

Chicken brain arylsulphatase A was purified by the method of FAROOQUI and BACHHAWAT (1972).

RESULTS

The product of the reaction using [^{35}S]cerebroside-3-sulphate as substrate was identified as inorganic sulphate by paper electrophoresis as described above and by paper chromatography as described earlier (FAROOQUI and BACHHAWAT, 1972).

Non-ionic detergents like Tween 20, Tween 80 and Brij 96 at 0·2 per cent concentration activate the enzyme activity approximately three-fold when cerebroside-3-sulphate was used as substrate. These detergents also activated slightly the enzyme activity towards *p*-nitrocatechol sulphate as substrate. Sodium taurodeoxycholate (0·2 per cent) has no effect on cerebroside-3-sulphate degradation whereas deoxycholate at the same concentration inhibits the enzyme activity to half of its original activity towards this substrate.

The enzyme showed maximum activity towards cerebroside-3-sulphate at pH 4·5 in acetate buffer. The pH optimum for *p*-nitrocatechol sulphate degradation in acetate buffer was 5·5 (FAROOQUI and BACHHAWAT, 1972).

The enzyme shows linear increase in [^{35}S]sulphate formation up to 60 min with cerebroside-3-sulphate as substrate and then the curve levels off (Fig. 1). This linear time–activity relationship has also been observed by HARINATH and ROBINS (1971) from human brain arylsulphatase A using 4-methylumbelliferone sulphate.

There was a linear increase in sulphate formation up to 0·3 mM concentration of cerebroside-3-sulphate. However, at 0·55 mM there was a decrease in enzyme activity. The approximate K_m and the V_{max} for both cerebroside-3-sulphate and *p*-nitrocatechol sulphate degradation are shown in Table 1.

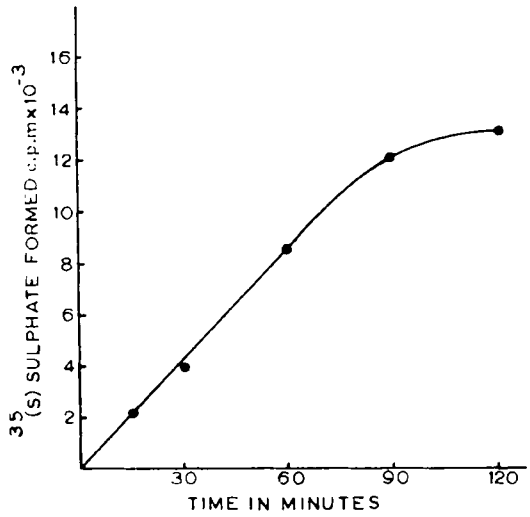

FIG. 1.—The time-course of [^{35}S]sulphate formation. Assay conditions were the same as described in the text except that the incubation period was varied as shown in the figure.

The relative rate at which the enzyme cleaves cerebroside-3-sulphate in 1600 times less than the rate at which it cleaves *p*-nitrocatechol sulphate. MEHL and JATZKEWITZ (1968) also observed a relatively low rate of degradation of cerebroside-3-sulphate by the pig kidney arylsulphatase A.

TABLE 1.—SOME KINETIC PARAMETERS FOR THE CHICKEN BRAIN ARYLSULPHATASE A

Parameter	Cerebroside-3-sulphate	*p*-Nitrocatechol sulphate*
pH	4·5	5·5
K_m (mM)	0·12	0·81
V_{max} (nmol/min/mg)	1·5	2500

* FAROOQUI and BACHHAWAT (1972).

The degradation of cerebroside-3-sulphate by chicken brain arylsulphatase A is inhibited by sulphite, phosphate and sulphate ions. Sulphite ions were powerful inhibitors compared to phosphate and sulphate ions. They almost completely inhibited the enzyme activity at 0·5 mM, while phosphate and sulphate ions at 1 and 2 mM inhibited the enzyme activity by 82 and 54 per cent respectively. MEHL and JATZKEWITZ (1964) found that pig kidney cerebroside sulphatase is inhibited by sulphite, phosphate and sulphate ions.

DISCUSSION

In the present study it was demonstrated that the purified chicken brain arylsulphatase A can degrade cerebroside-3-sulphate without any complementary heat-stable factor. MEHL and JATZKEWITZ (1968) have reported that arylsulphatase A from pig kidney can degrade cerebroside-3-sulphate and this degradation of cerebroside-3-sulphate is stimulated from seven to ten-fold by a complementary heat stable fraction. Recently JATZKEWITZ (1971) has reported that complementary heat-stable factor is required only at high buffer concentration (0·2 M) for cerebroside-3-sulphate degradation.

Like pig kidney cerebroside sulphatase (MEHL and JATZKEWITZ, 1968), chicken brain arylsulphatase A has the same optimal pH value, 4·5 and almost same K_m value, 0·12 mM. It is interesting to note that with cerebroside-3-sulphate as substrate, the enzyme does not show the anomalous time–activity relationship as shown by the enzyme when *p*-nitrocatechol sulphate was used as substrate (FAROOQUI and BACHHAWAT, 1972). These differences in the kinetic properties of arylsulphatase A towards different substrates may be attributed to the nature of the substrate.

Acknowledgements—We thank Dr. H. JATZKEWITZ of Max-Planck Institute, Germany and Dr. A. S. BALASUBRAMANIAN for helpful discussions. The work was supported by Grant No. NIH-01-024-1 from the National Institutes of Health, U.S. Public Health Service. A.A.F. is a Senior Research Fellow of the Council of Scientific and Industrial Research, India.

Neurochemistry Laboratory,
Dept. of Neurological Sciences,
Christian Medical College Hospital,
Vellore, India

A. A. FAROOQUI
B. K. BACHHAWAT

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