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Studies of Acetylcholinesterase in Synaptosomes

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Studies of Acetylcholinesterase in Synaptosomes

(TITLE)

BY

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Hyderabad, India

THESIS

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ABSTRACT

Acetylcholinesterase from rat brain was studied in crude homogenates, partially purified enzyme fractions, and in synaptosomes, to observe modification of the enzyme as a consequence of the modification of the membrane containing it.

Sodium dodecyl sulfate decreases the absorbance due to particles in the homogenate. This change in absorbance was proportional to both the concentration of sodium dodecyl sulfate and the concentration of the particles. Further, sodium dodecyl sulfate caused sulfhydryl groups to appear in the crude homogenate and in partially purified enzyme fractions. Synaptosomal preparations did not give such results. At concentrations of sodium dodecyl sulfate sufficient to cause absorbance changes, and therefore to modify the membranes in the preparation, the enzyme acetylcholinesterase was completely inhibited.

A non-ionic detergent in the same range of concentrations as for sodium dodecyl sulfate, neither inhibited the enzyme nor caused any noticeable change in absorbance due to the membranous particles.

INTRODUCTION

There are millions of nerve cells in the brain which require special enzymes for the synthesis and metabolism of substances which allow communication between cells. Nerve impulses are transmitted from one cell to another through a synapse. The transmission of information across the synapse occurs by the release of transmitter substance from the presynaptic membrane and its interaction with the receptor in the postsynaptic membrane (1). Acetylcholine is one of several transmitter substances involved in the transmission of information across synapses. Interaction of acetylcholine with its receptor in the postsynaptic membrane leads to excitation of the cell whereupon the acetylcholine is destroyed by hydrolysis catalyzed by the enzyme acetylcholinesterase (2). The components of neuronal membranes are important for understanding of the structure and function of this membrane.

Proteins are obviously important in the metabolism of neurotransmitters, the structure of the membranes, as well as other vital components of the chemical transmission from one cell to another. Several experiments have confirmed the classical observation of Weiss and Hiscoe (1948) (3) that cytoplasmic components may migrate down the axon. There are data suggesting that proteins may be also synthesized in

cytoplasmic and mitochondrial components of axons (3).

The lipid classes present in synaptic plasma membrane are very similar to whole brain (4). The rat synaptic plasma membrane is about two times richer in lipids than plasma membranes from rat liver. The most striking feature of synaptic plasma membrane is the higher proportion of longer chain fatty acids with a high degree of unsaturation in the synaptic plasma membrane than in whole brain. These fatty acids are primarily moieties of phosphatidylethanolamine and phosphatidylserine (4).

It is assumed that membrane bound proteins interact directly with some of the membrane lipids and are involved with the lipids in making up the structural matrix of the membrane (5). Since the hydrophobic groups of the proteins are thought to penetrate into the interior of the membrane, this interaction would lead to the conformation of the proteins that accommodates these deep associations (6). The important consequence of the physical arrangement of lipids and proteins in synaptic plasma membrane is that the presence of long chain unsaturated fatty acids would lead to steric restrictions on the conformation of the membrane bound proteins.

The organization of the synaptic region is rather complex (7). Of particular interest are the thickenings of the synaptic membranes, that is the presence of the intersynaptic filaments which join the two membranes across the cleft; and a system of filaments, the presynaptic web,

projecting into the postsynaptic region. Such components constitute what may be called the junctional complex of the synapse (7).

Most of the acetylcholinesterase is membrane bound and can be isolated in synaptosomes (3). Synaptosomes are artificially produced during homogenization by a closing of synaptic membranes to form small globules containing most of the junctional complex of the synapse (3).

Solubilization of proteins that are loosely associated with membranes can be done partially, or selectively, by methods that involve chelating agents, manipulation of ionic strength and pH. These methods, however, do not lead to solubilization of all proteins, especially those which are more strongly bound to the lipid matrix of membranes. For such proteins, the use of detergents serves the best purpose of solubilization (8).

As the concentration of the detergent increases in a suspension of liposomes, detergent is incorporated into the bilayer and hence causes changes in its physical properties by forming mixed micelles. The formation of mixed micelles gives rise to phase transitions after which increase in the detergent to phospholipid ratio causes a decrease in micelle size (8).

From the above knowledge, it is proposed that long chain unsaturated lipids and their deep penetration into the interior of the membrane give rise to hydrophobic interactions to form a sterically restricted structural

matrix containing the enzyme acetylcholinesterase. Slight disruption of the lipid matrix should force the enzyme into a different environment free of the original forces. In this new environment it is expected that the enzyme would exhibit altered chemical and physical properties.

Two possible ways to study the modification in the characteristics of the enzyme by modifying the membrane which holds it in a rigid conformation are either to solubilize the membrane and observe whether the enzyme was modified when released from the structural matrix of the membrane, or to treat the membrane with a detergent which would modify the membrane but keep the enzyme bound to the membrane.

We have attempted the latter. Using light scattering as a measure of the integrity of the synaptosomal membranes, we have sought conditions under which the membranes have been modified and under which the enzyme property of catalysis could be measured. We have sought changes in the catalytic properties of the enzyme acetylcholinesterase which could be traced to modification of the synaptic plasma membrane.

The colorimetric determination of acetylcholinesterase activity has been used by numerous researchers (9). Ellman (10) found that the product of enzymatic activity on acetylthiocholine (AcSCh) reacted rapidly with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to produce 5-thio-2-nitrobenzoic acid. At pH8, both of these compounds exist

as anions, or benzoates, rather than as free acids. 5-Thio-2-nitrobenzoate is highly colored with an extinction coefficient of E = 13,000 with a maximum absorption at 412 nm (yellow). Thus its rate of formation and therefore the rate of hydrolysis of AcSCh can be readily followed with a spectrophotometer. DTNB is a sulfhydryl reagent and the spectrophotometer determines the formation of -SH groups and thus the actual hydrolysis of AcSCh. AcSCh is a good substitute for the natural substrate (9).

The reactions involved are

(1) $H_2O + (CH_3)_3N^+CH_2CH_2S\overset{O}{\overset{\|}{C}}CH_3 \xrightarrow{\text{AChE}}$

$(CH_3)_3N^+CH_2CH_2S^- + CH_3COO^- + 2H^+$

(2) $(CH_3)_3N^+CH_2CH_2S^- + O_2N$-(C$_6H_3$(COO$^-$))-S-S-(C$_6H_3$(COO$^-$))-$NO_2$ $\xrightarrow[\text{non-enzymatic}]{\text{very fast}}$

$(CH_3)_3N^+CH_2CH_2$-S-S-(C$_6$H$_3$(COO$^-$))-NO_2 + O_2N-(C$_6$H$_3$(COO$^-$))-S^-

colored

MATERIALS AND METHODS

Special chemicals were obtained from the following sources: Sodium dodecyl sulfate (Eastman Organic Chemicals, Rochester, N.Y.); 5,5'dithiobis(2-nitrobenzoic acid) and acetylthiocholine (Nutritional Biochemicals Corporation, Cleveland, Ohio); 10% non-ionic detergent a polyol reagent No. 820 (Scientific Industries, Inc., Springfield, Mass.); all other chemicals were reagent grade. Rats were used from our laboratory colony.

Crude Homogenate:

All experiments were carried out at 4°C.

Rats were etherized, decapitated and the brains removed. A 10% homogenate was made in .32 M sucrose, .001 M EDTA and .1 M phosphate buffer. The brains were homogenized by seven up and down passes with a Thomas homogenizer using a teflon pestle.

Partially Purified Enzyme:

A 10% rat brain homogenate was made in .32 M sucrose, .001 M EDTA and .1 M phosphate buffer at pH8. This homogenate was centrifuged at 6000xg for 1 min using a Sorvall Superspeed centrifuge. The precipitate was discarded and the supernatant was centrifuged at 10,000xg for 10 min using a Beckman Model L3-50 Ultracentrifuge with the L-50

head. The supernatant was discarded and the precipitate was suspended in 1.5 ml of 0.32 M sucrose, .001 M EDTA and .1 M phosphate buffer per gram of brain.

Synaptosomal Preparation:

Partially purified enzyme was separated on a discontinuous sucrose gradient. A discontinuous sucrose gradient was prepared with 5.0 ml 1.2 M sucrose, 5.0 ml 1.0 M sucrose and .8 M sucrose. 1 ml of 1.5 ml/g of brain resuspension was carefully layered on top of the gradient and centrifuged at 25,000xg for 3 hrs in the L3-50 Ultracentrifuge using a SW 27.1 swinging bucket head. Synaptosomes at the interphase of 1.2 M and 1.0 M sucrose were eluted by displacing the contents with 2 M sucrose. Precautions were taken to avoid any bubbles that would mix up the gradient.

Enzyme Assay:

Acetylcholinesterase activity was determined by the method of Ellman (10) as described by Bennett (11). A stock solution of .4 M phosphate buffer was prepared by dissolving 104 g of $Na_2HPO_4 \cdot 7H_2O$ and 3.9 g of $NaH_2PO_4 \cdot 1H_2O$ in sufficient distilled water to make a final volume of 1 liter. This phosphate stock solution was diluted whenever needed to give .1 M phosphate which was adjusted to a pH of 8. To 2.8 ml of .1 M phosphate buffer .1 ml of enzyme was added and 50 µl of the DTNB reagent was delivered from a 50 µl "lambda" pipette. The contents were mixed in

the Cuvett and it was placed in the Bausch & Lomb Spectronic 70 set at 412 nm. The absorbance was adjusted to 0 absorbance units. To this 50 µl of AcSCh solution was added. The contents were rapidly mixed and replaced in the cell compartment of the spectrometer. Absorbance was recorded at thirty second intervals over a 5 to 7 min period.

The DTNB reagent was prepared by dissolving 40 mg of 5,5'-dithiobis-(2-nitrobenzoic acid) in 10 ml of .1 M phosphate buffer, plus 15 mg of sodium bicarbonate ($NaHCO_3$). AcSCh was prepared by dissolving 270 mg of acetylthiocholine iodide in 25 ml of distilled water. When 50 µl of this stock reagent is used in an assay with a final volume of 3 ml the final AcSCh concentration is $6x10^{-4}$ M.

Detergent Treated Pellet:

Both synaptosomal fraction and the partially purified resuspensions were used. Appropriate quantities of these preparations were mixed with .32 M sucrose, .001 M EDTA and .1 M phosphate at pH8 in the L-50 head tubes to give .1 ml of sample per 3 ml of solution. Two tubes containing this solution were centrifuged: one was treated with the detergent and the other was not. The centrifugation was carried out at 10,000xg for 10 min. The supernatant was saved in a test tube and the pellet was resuspended in .32 M sucrose, .001 M EDTA and .1 M phosphate buffer to give .1 ml sample per 3 ml of solution (Fig. 1).

RESULTS

Crude Homogenate:

The rate of the acetylcholinesterase reaction in the crude homogenate was determined at 5 different concentrations of AcSCh. Three trials were done with each concentration of substrate used. The enzyme follows Michaelias-Menten kinetics. When [v] is plotted against [s] a parabolic curve is obtained. A Lineweaver-Burke plot gives a straight line with an intercept of 1.85 min/mM and $-\frac{1}{Km} = -11.5 \times 10^{3}$/mole. V_{max} was calculated to be .54 mM/min and Km was found to be $.86 \times 10^{-4}$ M (Fig. 2).

SDS was found to inhibit acetylcholinesterase in the 10% homogenate (Fig. 3). The SDS gave 90% inhibition at a concentration 1.04×10^{-3} M and about 10% inhibition at 4.33×10^{-4} M. Incubation for about 10 minutes with 1.04×10^{-3} M SDS gave complete loss of activity. From Fig. 3 it can be seen that inhibition increases sharply in the range of 6×10^{-4} M SDS and 1.0×10^{-3} M SDS. The range of concentrations studied were .0125% and .05% which correspond to 4.33×10^{-4} M SDS and 1.733×10^{-3} M SDS.

The non-ionic detergent in the same range of concentrations had no effect on the rate. Therefore we thought that it may be the ionic character of the SDS causing a change in the ionic strength of the solution. The ionic

strengths of both the phosphate buffer and 6.93×10^{-3} M SDS were calculated from the following formula.

$$\text{Ionic strength} = \tfrac{1}{2} \sum M_i Z_i^2$$

where M is the molarity and z is the charge. This gave us the ionic strength of the buffer equal to .2843 at pH8 and that of 6.93×10^{-3} M SDS equal to .00693. Since the range of concentrations of SDS we were using were much lower than this, the ionic strength of the solution is not the factor which was causing inhibition.

Three different concentrations of 10% homogenate were studied with different concentrations of SDS. The concentrations of 10% homogenate studied were .15 ml, .1 ml and .05 ml and the range of concentration of SDS was same as above. When buffer, homogenate and water was used as a blank against buffer, homogenate and SDS it was found that the absorbance rapidly decreased and eventually stopped decreasing. The change was proportional to the concentration of SDS and the homogenate. This change in absorbance was taken as a measure of SDS modification of membranes in homogenate. When DTNB was added to a mixture of buffer, homogenate and SDS the absorbance jumped from zero to .44 absorbance units within 30 seconds and kept increasing slowly to .6. This change was proportional to both the concentration of homogenate and SDS. Since reaction

with DTNB is due to free sulfhydryl groups, this observation could be taken as a measure of the modification of the membranes in the preparations.

Synaptosomal Preparation:

A similar set of experiments was done on the isolated synaptosomes from the sucrose gradient. The Lineweaver-Burke plot of substrate variation gave V_{max} equal to .04901 mM/min and Km equal to $.47 \times 10^{-4}$ M. Complete inhibition of acetylcholinesterase was observed at even the lowest SDS concentration used above (4.33×10^{-4} M SDS). When the SDS concentration was reduced to lower levels a point was reached where there was little inhibition. However at this low level of detergent no measurable effect on the absorbance due to the membranes or due to reaction with DTNB could be detected. Consequently it was deemed impossible to measure modification of the membranes by these methods.

The inhibition by the non-ionic detergent at higher concentrations in synaptosomes shows that at .167% non-ionic detergent concentration there was 18% inhibition, that at about 1.34% non-ionic detergent concentration there was about 84% inhibition and that 1.67% gave 100% inhibition. The inhibition was found to increase linearly with the concentration of non-ionic detergent (Fig. 4).

The following experiment was done on synaptosomes to determine if the enzyme was released into the supernatant

by detergent treatment. Synaptosomes treated with 4.33×10^{-4} M SDS were subjected to 10,000xg for 10 min. Control synaptosomes not treated with SDS were also centrifuged. Both the SDS-treated synaptosomes and those not treated with SDS gave about 60% enzyme activity in the supernatant. Since phosphate buffer was used as the suspension medium and lysis of synaptosomes could occur, we changed the suspension medium to the homogenizing medium at pH8 to give stability to the membrane bound enzyme during centrifugation. This change from .1 M phosphate buffer to the homogenizing medium as resuspension medium did not alter the results.

The pellet treated with SDS and non-ionic detergent, and the control pellet (not treated with any detergent), in the synaptosomal preparations gave 100 per cent activity in P_a and S_a.

Partially Purified Enzyme:

A similar experiment was done on the partially purified enzyme. Partially purified enzyme, here onwards referred to as P, was subjected to treatment with detergent and centrifuged at 10,000xg for 10 min, to give a supernatant and a pellet. The above supernatant is referred to from here onwards as P_a and the pellet which was resuspended in .32 M sucrose, .001 M EDTA and .1 M phosphate buffer at pH8 is referred to here onwards as P_b. Partially purified enzyme which was not treated with any detergent was used as a

control and subjected to centrifugation at 10,000xg for 10 min which gave supernatant and a pellet. The resultant supernatant will be called S_a; and, the pellet resuspended in homogenizing medium will be called S_b.

When P was treated with 1.733×10^{-3} M SDS about 5% enzyme activity was found in P_b and the rest was presumably solubilized. When P_a was analyzed for the enzyme, it was noticed that, just by the addition of DTNB to a 2.9 ml aliquot of supernatant P_a, the absorbance increased rapidly to .4 and from then on increased slowly to a maximum of .43. This was adjusted to zero absorbance. Addition of AcSCh at this stage resulted in a linear increase in absorbance equivalent to about 4% enzyme activity.

Treatment of P with 4.33×10^{-4} M SDS gave 60% activity in P_b. When 50 µl of DTNB was added to a 2.9 ml P_a there was a rapid increase in absorbance which reached .19 absorbance units then slowly increased to .22 absorbance units. This was adjusted to zero absorbance. Addition of AcSCh gave a linear increase in absorbance which was equivalent to 38% activity. With the control it was found that there was 93% activity in S_b and 8% in S_a. There was no increase in absorbance when the homogenizing medium was treated with SDS and DTNB (Table 1).

Partially purified enzyme when treated with .67% non-ionic detergent, gave 5% of the enzyme in P_a and 95% in P_b. A 1.34% non-ionic detergent gave 95% activity in P_b and an unidentifiable contaminating activity in P_a,

which was due to interaction between the homogenizing medium, non-ionic detergent and DTNB.

DISCUSSION

The kinetic constant K_m and V_m calculated for our crude homogenate differ relatively little from the values reported on whole brain by Ellman (10). The enzyme activity calculated from our data gave a value of 16.76×10^{-8} m moles/min/mg of brain compared to that of 67.6×10^{-7} m moles/min/mg of brain determined by Bennett (11). As a result of an extensive study on rat brain, blood and intestine it was shown that only about 5% activity in preparations similar to ours was due to a non-specific enzyme cholinesterase (9), and that crude membrane preparations obtained from disrupted synaptosomes have approximately 80 per cent of the acetylcholinesterase activity of the intact synaptosomes (12, 13). Therefore our assay conditions gave authentic acetylcholinesterase activity from the synaptosomes and initially this enzyme activity is membrane bound.

From our experiments we have demonstrated that sulfhydryl groups are released by SDS in whole brain homogenates and in the partially purified enzyme. When the same concentrations of SDS were used with the purified synaptosomes, no sulfhydryl groups could be detected.

The main reason sulfhydryl groups could not be detected at low SDS concentrations with the synaptosomes is that any released would be at a concentration too low

to detect. It might be anticipated that release of the enzyme acetylcholinesterase from the membrane might be accompanied by an appearance of sulfhydryl groups. However, the content of sulfhydryl groups in the enzyme is in dispute (14, 15, 16, 17). Lauzinger (15) has reported that acetylcholinesterase from electric eel splits into four subunits in presence of guanidine and mercaptoethanol. Since the enzyme splits into four subunits it can be suggested that there might be intrasubunit disulfide bridges to hold the tertiary structure of the enzyme. Froede and Wilson (17) have reported that SDS in low concentrations produces inactive subunits with $S_{20,w}$ Values between 3.2 and 4.73. These results suggest that there are no intersubunit disulfide bridges. The concentration of SDS which produced inactive subunits was not reported.

Therefore our results from synaptosomal preparations are consistent with an enzyme having few sulfur containing moieties with no disulfide bridges involved in the quaternary structure.

Since the synaptosome preparation had, at most, 3 mg protein/ml, assuming that there were 20 m mole of sulfhydryl for each 233,000 mg protein, and since the molar absorbtivity of 5-thio-2-nitrobenzoate is 13,600, then the greatest absorbance we could expect would be 0.100 after reaction with DTNB. However, this assumes that 100% of the protein present is acetylcholinesterase. Since a reasonable expectation would be that the enzyme makes up less

than 10 per cent of the protein, it is concluded that the sulfhydryl groups from the enzyme would be undetectable by these methods.

The centrifugation studies on the partially purified resuspension and synaptosomal fraction resuspension were done to determine whether or not the enzyme was being solubilized from the membrane. Indeed there was a difference found between the pellet from the partially purified enzyme which behaved normally and the supernatant which gave very little activity but released sulfhydryl groups.

In contrast to the partially purified enzyme, the synaptosomal preparation produced no pellet on centrifugation, whether treated with detergent or not.

It was impossible to reach definite conclusions because of the following difficulties:

1) The purified synaptosome preparations contained a low amount of enzyme activity.
2) The non-ionic detergent at lower concentrations did not release the enzyme from the membrane. At higher concentrations, the non-ionic detergent instead of releasing the enzyme from the membrane, gave a contaminating absorbance in the supernatant P_a, therefore the validity of the observations are doubtful in presence of this non-ionic detergent.
3) The synaptosomal preparation was completely inhibited by very low concentration of SDS (4.33×10^{-4} M).
4) The synaptosomal preparation when treated with a

non-inhibitory concentration of SDS gave 100 per cent activity in both P_a and S_a.

The following few points are suggested for further investigations to accomplish definitive results.

Since the purified synaptosomal plasma membrane from sucrose gradient contains low level of enzyme activity, a Ficoll-sucrose discontinuous density gradient would give much better preparations (12). From a recent report it was found that the crude homogenate had 80 per cent of the acetylcholinesterase activity of the intact synaptosomes and approximately 50 per cent is recovered in the synaptosomal plasma membrane purified by sucrose density gradient.

The mode of action of SDS is a cooperative association to all proteins and the maximum amount of SDS that is bound per gram of protein is the same for most of them (6). The interaction of SDS with most proteins is hydrophobic, which can be seen from the following line of evidence. Seventy per cent of the SDS bound to Cytochrome b binds to the tryptic fragment, yet the tryptic fragment represents only 30 per cent of the protein. The tryptic fragment of Cytochrome b contains the hydrophobic region of the protein (18). The result of the cooperative binding of SDS to proteins having quaternary structures usually is dissociation of them into their constituent polypeptide chains. Therefore SDS would not be a good detergent for an enzyme which is susceptible to dissociation at low concentrations.

A comparative study of the non-ionic detergent

Triton X-100 against SDS or non-ionic detergent polyol favours the use of Triton X-100 for the following reasons. The binding of the non-ionic detergent Triton X-100 to a number of water soluble proteins has been studied and no binding to sites other than the high affinity sites of serum albumin has been detected (8). Triton X-100 neither appears to induce conformational changes in proteins leading to loss of their biological properties nor does it allow denaturation so that proteins can preserve their quaternary structures in presence of high concentrations (8).

The nature of the charged head group and the length of the alkyl tail of a detergent are important and influence both the critical concentration needed to induce cooperative binding and the resulting conformational change. Since Triton X-100 has rigid and bulky apolar moieties, it probably does not penetrate crevices of the protein surfaces as efficiently as the flexible alkyl chains of other detergents (8).

This detergent has been used by several researchers to solubilize the membrane bound acetylcholinesterase. One report says that more than 90 per cent of the homogenate activity was recovered in the initial detergent supernatant in contrast to only 10-12 per cent in the residue (19). The concentration of this detergent used for solubilization was .1 per cent. This concentration does not inhibit acetylcholinesterase (12). In the light of the above information it can be said that sodium dodecyl sulfate which has a

flexible alkyl chain penetrates efficiently into the crevices of proteins making cooperative associations hence breaking the enzyme into inactive subunits. The non-ionic detergent, a polyol, used in this study might have a branched alkyl tail which could not penetrate into the crevices of protein surfaces at all therefore remained inactive in breaking up the lipid bilayer.

Hence it is proposed that for further studies Triton X-100 might give better results based on the fact that it preserves the quaternary structure of protein.

A new technique to understand induced conformational changes has been developed by Gotto and Kon (20). The procedure requires spin-labeling which is very sensitive and enables one to draw definite conclusions.

Spin labels have been employed to study the effect of phospholipase C action on the molecular motion of the phospholipids in nerve membranes (21). Electron spin resonance methods were also used to investigate the effects of independent variation of the lipid and protein composition on the organization of the lipid in viral membranes (22). The viruses were labeled with nitroxide derivatives of stearic acid.

From the studies on the conformation of human serum high-density lipoproteins, it was concluded that electron paramagnetic resonance is more sensitive than infrared spectroscopy or circular dichroism to changes in local environment. The lipid-protein interactions in high-density

lipoproteins were examined by labeling the free amino groups of the protein moieties with a maleimide nitroxide derivative (20). It was inferred that signals were very sensitive to changes in the local environment of the nitroxide radical which may reflect overall alterations in the conformation of the protein (20).

It is finally proposed that by incorporation of nitroxide derivatives of stearic acid into the phospholipids or by labeling the free amino groups of proteins, the change in the membrane and consequently a change in enzyme can be followed by partial solubilization of membrane bound enzyme by detergents. The difference in environment of a protein ie when bound to membrane would give a signal corresponding to a specific conformation in the lipid bilayer which would be different when the protein is solubilized and hence in a different conformation corresponding to the new surroundings.

TABLE I

P = .57 Δ od/min = 100% activity

S_a = .05 Δ od/min = 8% activity

S_b = .535 Δ od/min = 93% activity

	4.33×10^{-4} M SDS	1.733×10^{-3} M SDS
P_a	38% activity	2% activity
P_b	60% activity	5% activity

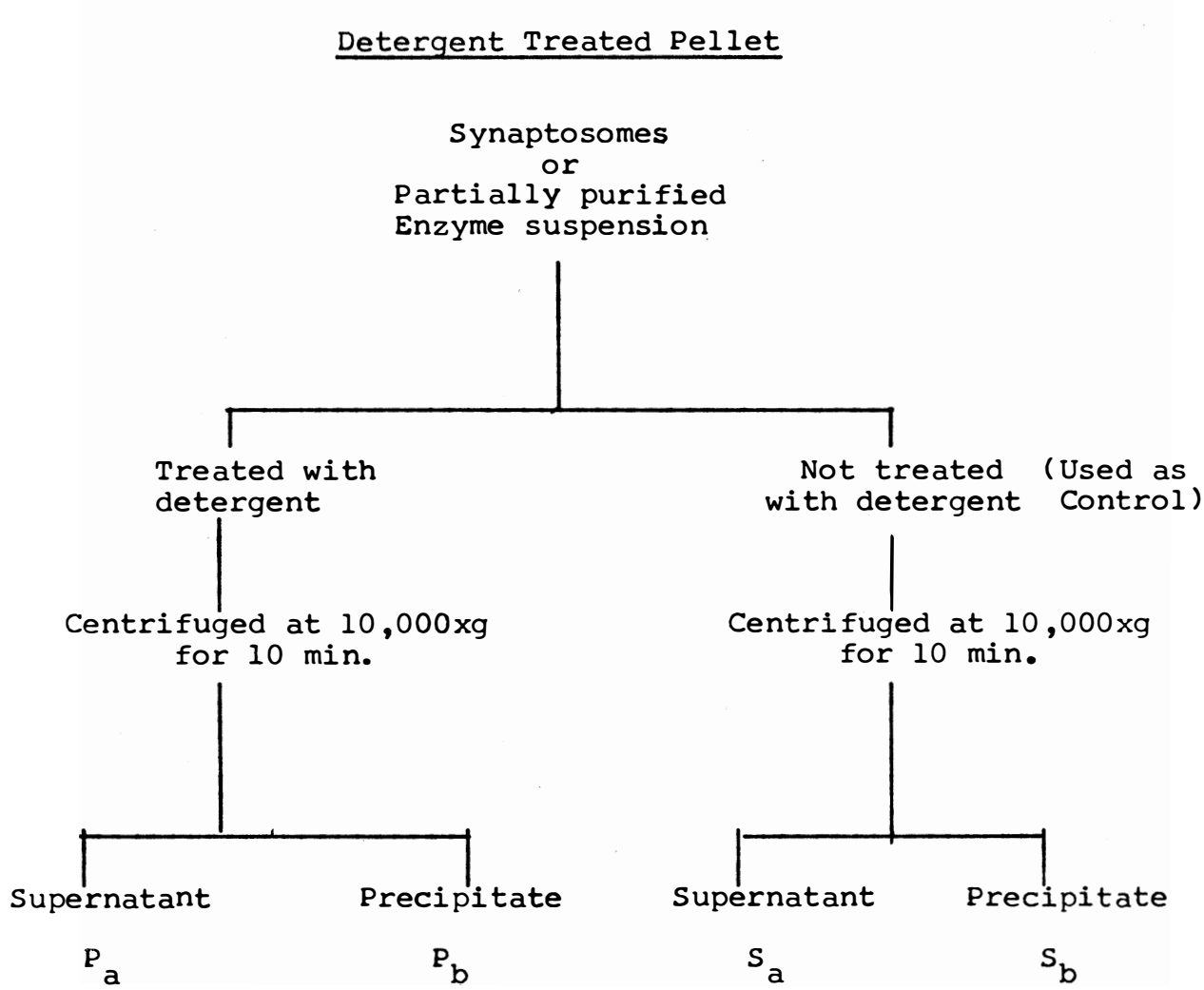
1.733×10^{-3} M SDS:

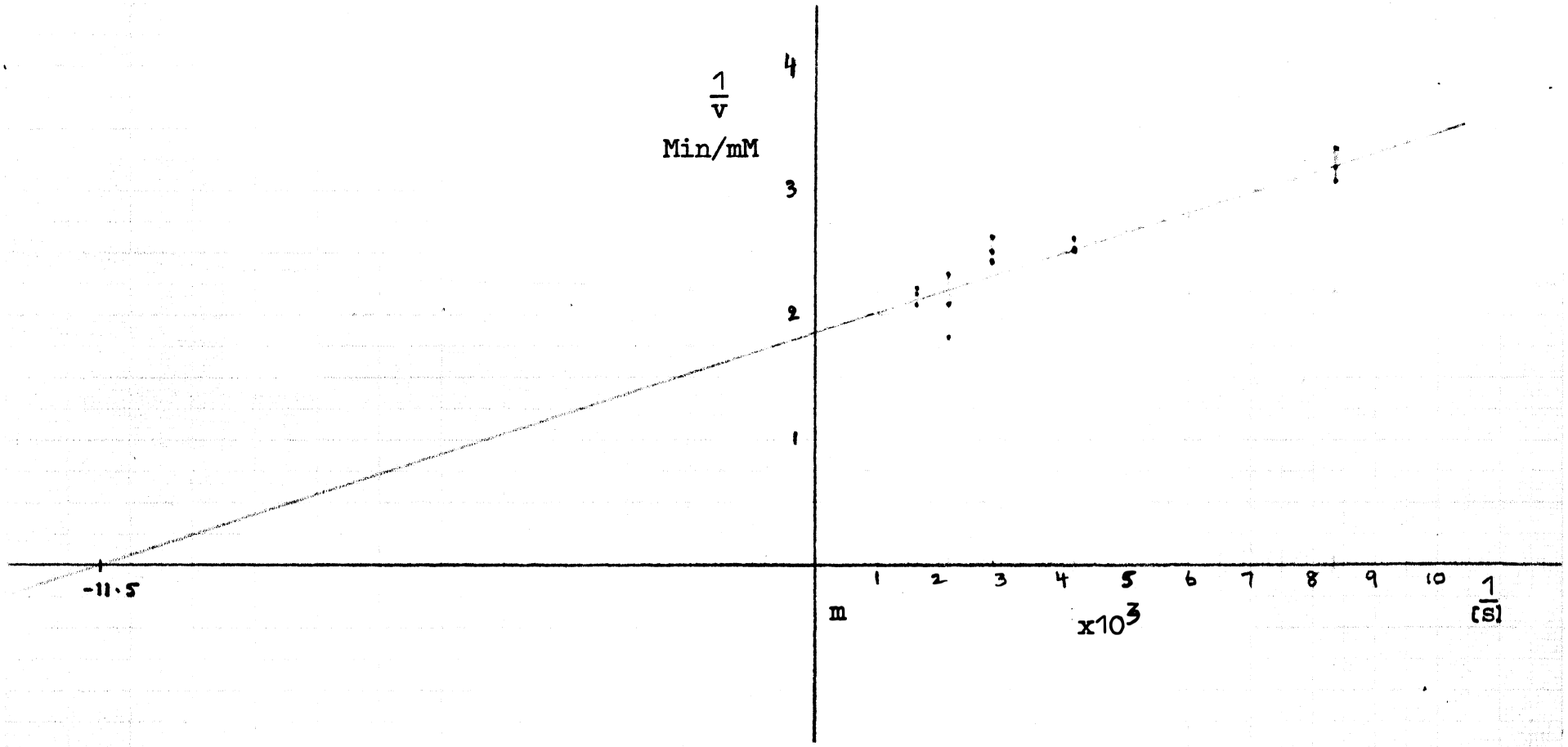
		% activity after addition of AcSCh
P_a + DTNB	.43 od units	2% activity

4.33×10^{-4} M SDS:

		% activity after addition of AcSCh
P_a + DTNB	.22 od units	38% activity

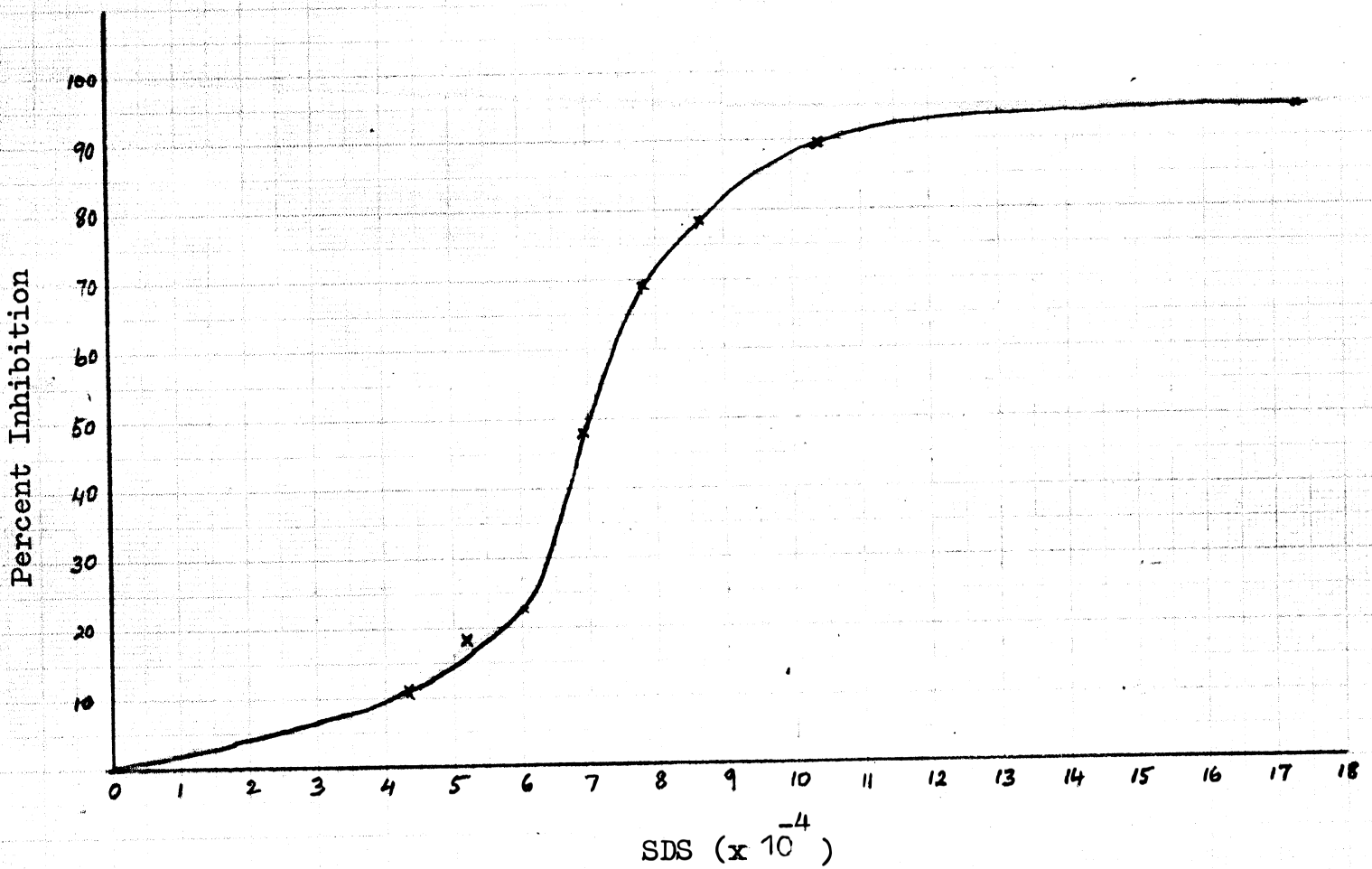
Fig. 1


Detergent Treated Pellet
Synaptosomes
or
Partially purified
Enzyme suspension
Treated with
detergent
Not treated with detergent
(Used as Control)
Centrifuged at 10,000xg
for 10 min.
Centrifuged at 10,000xg
for 10 min.
Supernatant
Precipitate
Supernatant
Precipitate
P_a
P_b
S_a
S_b



$\frac{1}{V_m}$ = 1.85 Min/mM

$\frac{-1}{K_m}$ = -11.5 liter/mole


Percent Inhibition
100
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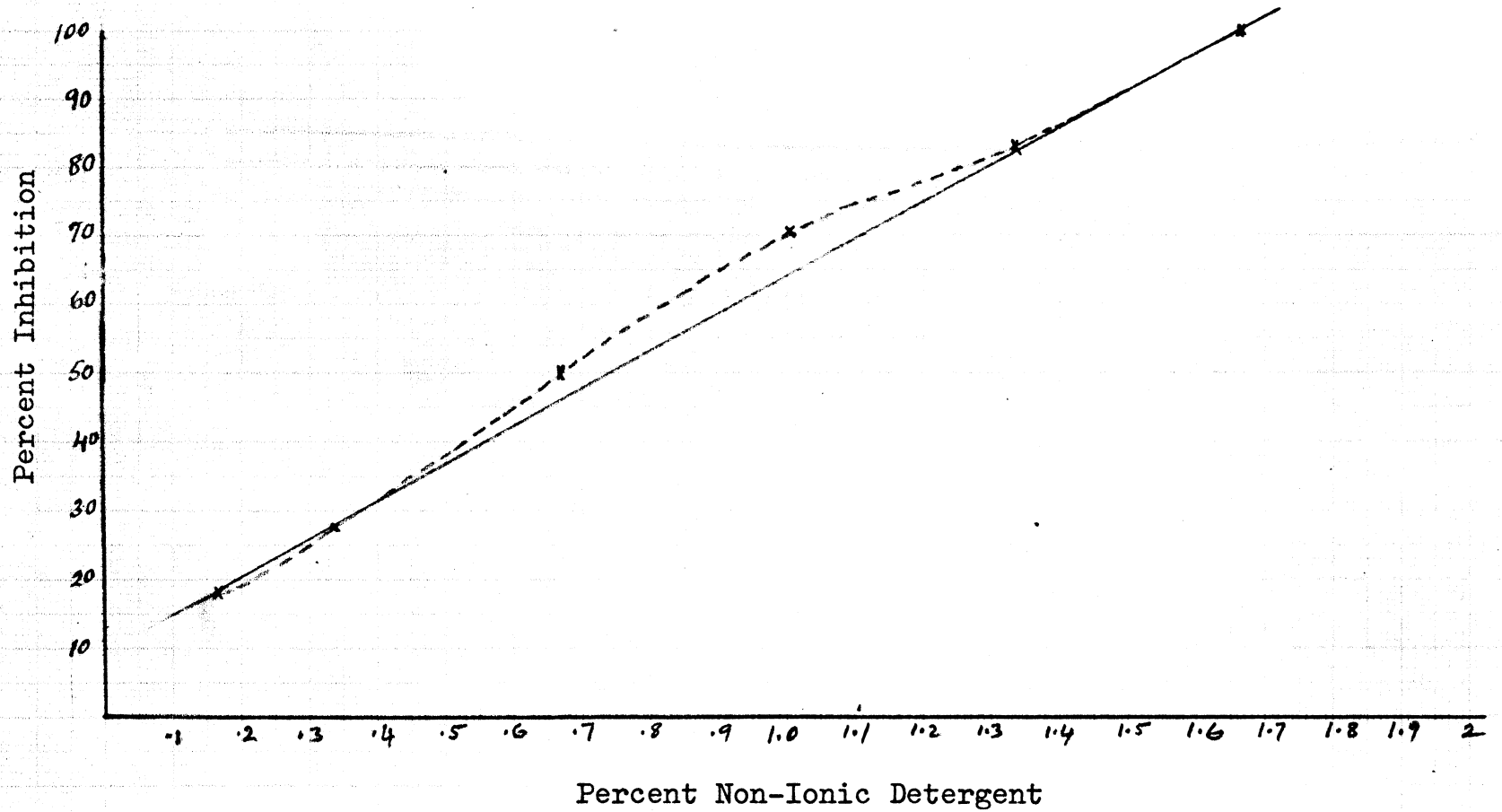
80
70
60
50
40
30
20
10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
SDS (x 10^{-4})


Percent Inhibition
100
90
80
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1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2
Percent Non-Ionic Detergent

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