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ENTHALPY OF ACETYLCHOLINE HYDROLYSIS BY ACETYLCHOLINESTERASE *

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Direct microcalorimetric measurements were made of the reaction between acetylcholine chloride and acetylcholinesterase (EC 3.1.1.7) that was extracted from electric eel (*Electrophorus electricus*) and purified by affinity chromatography. Tris-HCl, sodium phosphate and potassium phosphate were used as buffers and sources of ions for the reaction. At pH 7.2 and in 0.1–0.2 M phosphate buffer, the ΔH for acetylcholine hydrolysis was found to be -0.107 kcal/mol (under buffered conditions) and -0.931 kcal/mol under unbuffered conditions (water). At pH 8.0 in 0.1 M Tris-HCl buffer, values greater than -2.5 kcal/mol were obtained, with the highest value of -9.2 kcal/mol being seen with bovine erythrocyte acetylcholinesterase. Tris-HCl buffer at 4×10^{-2} M enhanced the reaction velocity by 51.2% over that of 4×10^{-3} M buffer. Enzyme purity, pH and ionic milieu of reaction mixture, and substrate concentration affected the measured ΔH value.

1. Introduction

At the cholinergic synaptic junctions, the transmission of a nerve impulse is mediated by acetylcholine through its release from the presynaptic vesicles. Immediately following its action on the postsynaptic membrane, the neurotransmitter is effectively destroyed by a neural membrane-bound enzyme, acetylcholinesterase (EC 3.1.1.7), so that the neurons are restored to their resting stage. In view of the fact that acetylcholine is synthesized in the neural cytosol from the recycled choline, it has been hypothesized that the hydrolytic and synthetic reactions may be energetically coupled. Hence, an enthalpy value for acetylcholine hydrolysis would appear to be of importance in understanding this complex phenomenon.

Sturtevant [1] published the value of 280 ± 20 cal/mol for the enthalpy of hydrolysis of acetylcholine at pH 7, 25°C, in phosphate buffer. Several authors had calculated thermodynamic values for the hydrolysis and intermediate steps in this second-order reaction (Hestrin [2]: this value was recalculated by Browning [3], Wilson and Cabib [4], Lumry [5], and Rosenberry and Neumann [6]). Generally, the calculated thermodynamic values are limited by the difficulties implicit in obtaining binding constants with sufficient precision at multiple temperatures for enthalpy determination by the van't Hoff method. Also, calculation of values for events subtending the catalytic hydrolysis, which are always based upon assumption of a reaction model, remain themselves unsubstantiated hypotheses. Hence, such values are useful primarily as targets for experimental tests. Models used by these authors have been built upon extrapolation from the more extensive literature dealing with chymotrypsin.

Ultimately, thermodynamic reference values are best obtained from direct measurement. Refine-

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ments, in instrumentation and particularly in enzyme purification technology, have been made which allow a measurement to be made now. We report here the enthalpy of acetylcholine hydrolysis measured in conduction-type (pseudoisothermal) batch microcalorimeters. Various reaction media and enzyme sources have been used to obtain energetic relationships between various interacting molecules.

2. Experimental

2.1. Materials

Acetylcholinesterase was extracted from eel electric organ and purified by affinity chromatography in our laboratory according to the method of Massoulié and Bon [7].

Electrophorus electricus, purchased from Worldwide Scientific Animals (Opopka, FL), was shocked by immersion in crushed ice for 15 min. The electric organ was then removed and cut into small pieces (5 cm^3). Batches were then quickly chilled with liquid nitrogen and stored at −85°C until use.

Hexamethylenediamine-coupled polyacrylamide gel (HMD-Ultrogel AcA 34) was purchased from LKB. *m*-Carboxyphenyldimethylethyl ammonium iodide was synthesized using the method of Massoulié and Bon [7]. This product was then coupled with the gel using *N*-ethoxycarboxyl-2-ethoxy-1,2-dihydroquinoline, EEDQ (Sigma), in a water/ethanol mixture. 50 ml of the affinity gel was packed into a column (Pharmacia). Efflux was monitored using an LKB ultraviolet detector connected to a chart recorder.

The tissue pieces, removed from storage, were homogenized with an equal volume of cold distilled water for 2 min, then centrifuged at 20000 × *g* for 1 h. The pellet was resuspended and homogenized with 2 M NaCl solution, 2 ml/g pellet, for 5 min. It was then centrifuged at 26000 × *g* for 1 h. The supernatant was treated with ammonium sulfate at a rate of 300 g/l, 23°C for 15 min. Precipitated protein was obtained by centrifugation at 26000 × *g* for 30 min, suspended in 0.1 M Tris-HCl buffer and dialyzed overnight against a large volume of the same buffer. Flocculated material was removed by centrifugation at 26000 × *g* for 30 min and the resulting clear supernatant was used for column chromatography.

This enzyme extract was applied to the affinity column to saturation and then washed free from the excess extract with 3 column volumes of Tris-HCl buffer. Enzyme was eluted with 0.2 M tetraethylammonium in 0.1 M Tris buffer, pH 7.0. The enzyme solution containing tetraethylammonium was dialyzed for 24 h against a large volume of distilled-deionized water. The enzyme solution, believed to be salt-free, was placed into individual glass vials and freeze-dried for 12 h, after which the vials were capped in dry gaseous nitrogen and stored at 4°C.

Catalytic activity of the affinity-purified enzyme was assayed either photometrically using acetylthiocholine [8] or electrometrically using acetylcholine [9]. Protein concentration was determined using a modified Lowry method [10]. The product of this procedure yielded an activity of 7000–10000 U/mg.

2.2. Calorimetric measurements

Calorimetric measurements were made using bismuth telluride thermopile-based reaction microcalorimeters. These conduction-type instruments, constructed in this laboratory, are similar to the design published by Evans et al. [11]. Operated as a dual differential instrument, the experimental acrylic partition cell contained the reaction mixtures, a matched reference cell containing an equal volume of water. In a typical experiment, the enzyme in buffer was placed on one side of the reaction vessel and substrate in water on the other. Mixing was accomplished by rotating the instrument. Voltage output of the differentially arranged thermopiles was amplified by a chopper-stabilized d.c. amplifier (Analog Devices 261K) followed by an instrumentation amplifier of conventional design. The output voltage was recorded using a chart recorder and, after A/D conversion, input to a Hewlett Packard 9825T computer for data deconvolution and automated data analysis. Sensitivity of the instrument is sufficient to allow meas-

urement of heat changes in the low microcalorie range.

Thermal calibration was accomplished using Tris hydroxyaminomethane vs. hydrochloric acid neutralization [12] as standard. Reaction heats were measured at 27°C.

3. Results

3.1. Reaction medium

Ideally, the various molecules that compose the reaction medium should be water-soluble, in adequate ionic strength to maintain the enzyme activity and to provide adequate buffering, and not contribute significant ionization or protonation heats.

We first looked at the enzymatic hydrolysis of acetylcholine in a medium of distilled-deionized water (fig. 1). This 'ion-free' medium is obviously unsuitable for a normal catalysis under the usual unlimited substrate conditions. It is perhaps not impossible to make an accurate measurement in this medium if the substrate and enzyme are at stoichiometric levels and their concentrations are high enough to yield measureable heats. We observed that, if the medium is not buffered and the substrate is far in excess of the enzyme, the nature of the thermal output is altogether nonrepresentative of the hydrolytic reaction per se (fig. 2).

We have evaluated various buffers and buffer molarities with respect to their suitability for these measurements. Tris-HCl buffer at pH 8.0 was initially used to examine the reaction kinetics at various buffer strengths (fig. 3). In buffer molarity lower than 1 mM, the reactions do not go to completion. Acetylcholine hydrolysis in buffer concentrations of 4×10^{-3} and 4×10^{-2} M gave the same heat; the substrate is completely consumed in both cases and yielded the same total heat (area under the curve). However, at the higher buffer concentration, the reaction velocity was increased by 34% (peak height of the curve). Based upon these results and in line with the literature, we used 0.1 M Tris-HCl buffer as a medium for the reaction in later studies.

Sodium phosphate buffer, at 0.05, 0.1 and 0.2 M, reacted with 3 μmol acetic acid endothermically (fig. 4). These reactions were carried out at pH 7.2 in order to provide baseline values for enthalpy measurements of acetylcholine hydrolysis at near-neutral pH. This buffer is unlike both

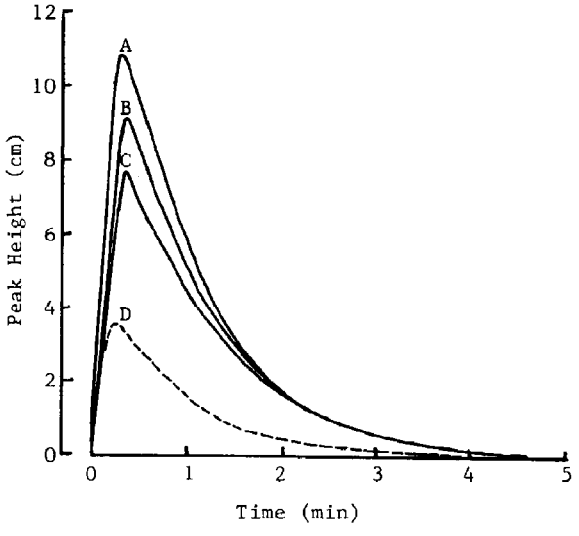

Fig. 1. Reaction heats between acetylcholinesterase (20000 U in 1.0 ml distilled-deionized water) and various concentrations of acetylcholine in 1.0 ml water. (A) 3.0 μmol (−1.5564 mcal), (B) 1.5 μmol (−1.2652 mcal), (C) 0.75 μmol (−1.0736 mcal), (D) blank (water).

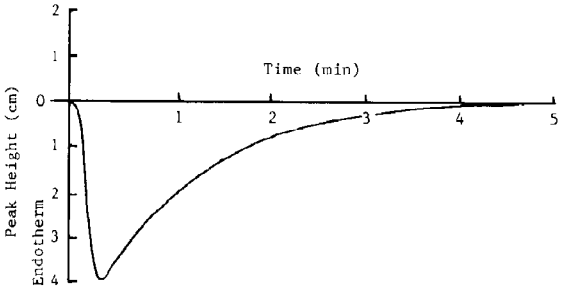

Fig. 2. Reaction heat between acetylcholinesterase and acetylcholine under nonbuffered and ion-free conditions. Acetylcholine (150 μmol in 1.0 ml) was used approx. 6000-times more than the enzyme active site stoichiometry 6700 U in 1.0 ml). The recorded endothermic heat was 7.7825 mcal or 0.052 kcal/ml acethylcholine introduced or 894.987 kcal/mol enzyme active sites.

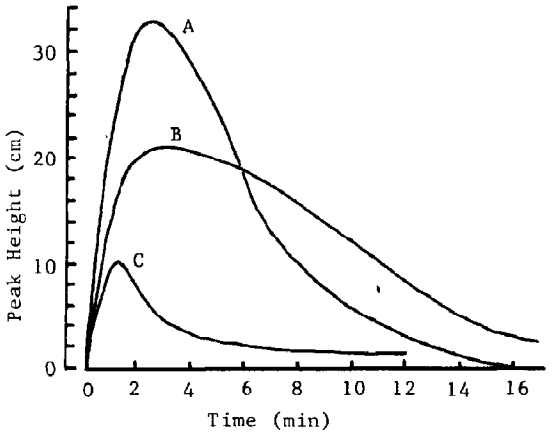

Fig. 3. Effect of buffer molarity (Tris-HCl, pH 8.0) on the reaction kinetics of acetylcholine hydrolysis by acetylcholinesterase. 2 U AChE in 1.0 ml buffer was reacted with 3 μmol ACh in 1.0 ml water (A) 4×10^{-2} M (−20.735 mcal), (B) 4×10^{-3} M (−20.915 mcal), (C) 4×10^{-4} M (> −4.375 mcal, incomplete measurement). Note the difference in reaction velocities (peak heights) between A and B (34%) although the total heat output was nearly the same in both cases.

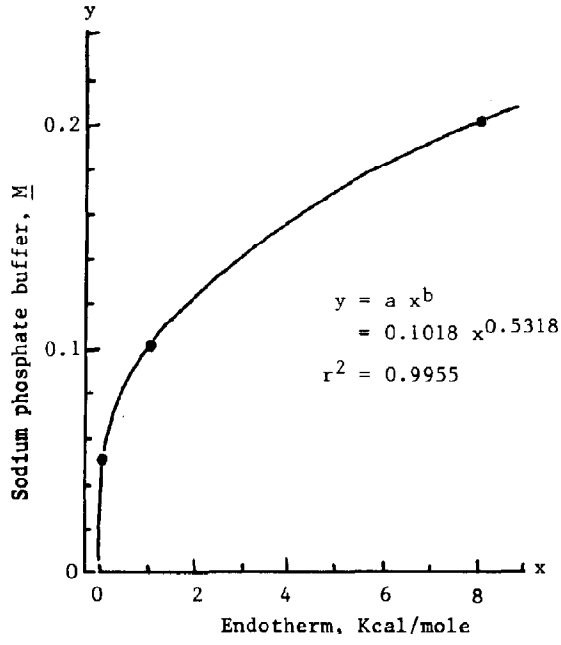

Fig. 4. Reaction heats between 3 μmol acetic acid in 1.0 ml water and various molarities of sodium phosphate buffer, pH 7.2 (1.0 ml). Heats, were corrected for buffer dilution with water.

Tris-HCl and potassium phosphate buffers in its reaction with acid (table 1). The logarithmic increase in the endotherm is suggestive of a salt-dilution effect peculiar to sodium and which is therefore unsuited to our reactions. Tris-HCl buffer did not exhibit any significant variations either as a function of the particular acid (acetic and hydrochloric) or as a function of concentration. However, the reaction heat in a Tris-buffered medium is strongly exothermic – the contribution of the buffer protonation heat [13–15]. Although the high protonation heat is often advantageous, particularly in analytic microcalorimetry, there is concern here that the large contribution of the buffer protonation to the measured heat would serve to obscure other less energetic events which are a part of the reaction sequence.

Potassium salts of phosphoric acid were ultimately our choice for these microcalorimetric measurements. They exhibited low exothermic protonation heats which were consistent regardless of the proton source or concentration (table 1).

3.2. Substrate concentration

It is known that acetylcholinesterase forms an inhibitory complex with acetylcholine and that the reaction velocity is reduced if the substrate con-

Table 1

Reaction heats between buffers and acid solutions in water

Buffer (1.0 ml)	Acid (μmol/ml)	kcal/mol acid *
Tris-HCl	hydrochloric acid	
0.1 M, pH 8.0	0.25	−14.520
	1.0	−13.592
	acetic acid	
	0.25	−14.214
	1.00	−11.523
Sodium phosphate	acetic acid	
0.2 M, pH 7.2	3.0	3.724
0.1 M, pH 7.2	3.0	0.874
0.05 M, pH 7.2	3.0	0.278
Potassium phosphate	hydrochloric acid	
0.1 M, pH 7.2	3.0	−0.945
	6.0	−0.824

* Corrected for heat of buffer dilution with equal amount of water (without acid).

centration exceeds approx. 10^{-3} M. For this reason, we carried out a number of reactions with varying substrate and enzyme concentrations. Typical reaction tracings are shown in figs. 5 and 6 for noninhibitory conditions and in fig. 7 for the inhibitory substrate concentrations. In the latter case, the peak height decreased and the baseline 'drifted' proportionately to the increase in substrate concentration. All these reactions were carried out in Tris-HCl buffer.

3.3. Instrumental output and data treatment

Voltage output from the calorimeter thermopiles track the process represented by the chemical or physical event within the vessel. The heat flow characteristics of the device impose a thermal impedance upon the output which makes it necessary to interpose a deconvolution procedure upon the data before it can be considered to reflect the process kinetics accurately. Following the logic of Calvet and Prat [16], however, thermodynamic values are directly recoverable from the area under the calorimeter output tracing. Although some error, in theory, results from losses from vessel surfaces which are not in thermal contact with the

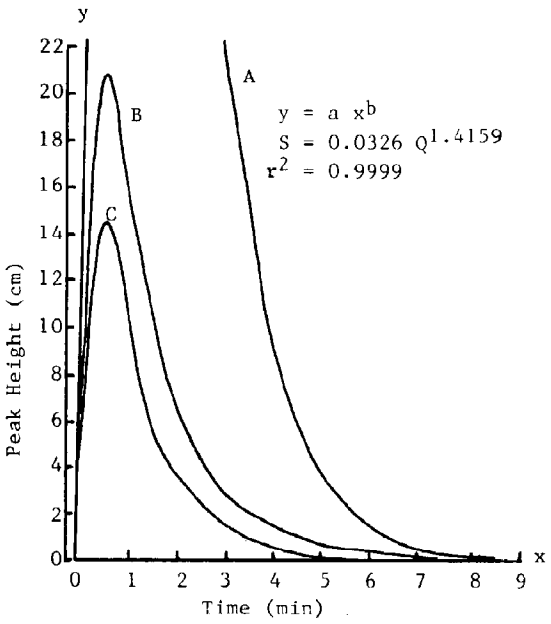

Fig. 6. Reaction heats between 2.5 U of acetylcholinesterase in 1.0 ml of 0.1 M Tris-HCl buffer, pH 8.0, and various concentrations of acetylcholine in 1.0 ml water. (A) 3.03 μmol (−24.610 mcal), (B) 0.606 μmol (−7.821 mcal), (C) 0.303 μmol (−4.855 mcal). Heat output (Q) was exponentially linear to the substrate concentration (S).

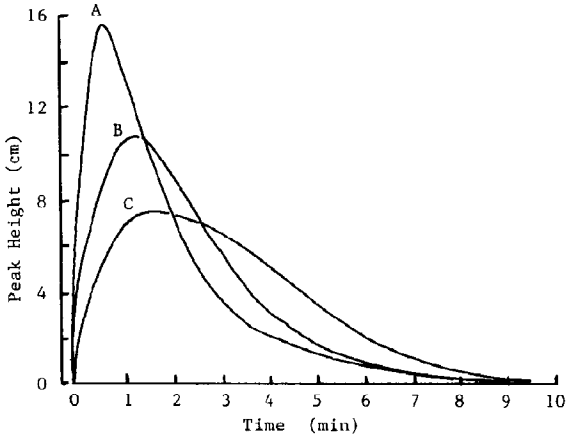

Fig. 5. Reaction heats between 0.303 μmol acetylcholine in 1.0 ml water and various amounts of acetylcholinesterase in 1.0 ml of 0.1 M Tris-HCl buffer, pH 8.0. (A) 1.0 U (−6.358 mcal), (B) 0.3 U (−6.811 mcal), (C) 0.1 U (−7.068 mcal). Note the increase in velocity (peak height) with the increase in enzyme concentration.

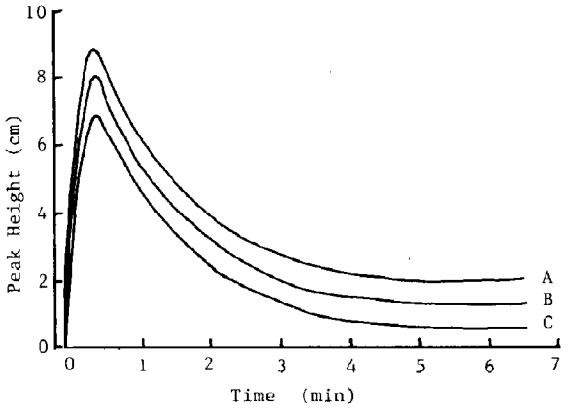

Fig. 7. Reaction heats between acetylcholinesterase (0.01 U in 1.0 ml of 0.1 M Tris-HCl buffer, pH 8.0) and various concentrations of acetylcholine in 1.0 ml water. (A) 0.152 mM, (B) 0.303 mM, (C) 0.606 mM. All reactions were incomplete. Peak heights and baseline drifts were inversely proportional to the substrate concentrations.

thermopiles, such losses are probably negligible for the instruments used here. In any case, calibration values within the measurement range establish that heat output is a linear function of the thermal output of the reaction. Hence, an integration of the values obtained, millivolts/time, is proportionate to Q, the quantity of heat required by a system for a change from state 1 to state 2 under a constant external pressure [1]:

$$Q = (E_2 + PV_2) - (E_1 - PV_1) = H_2 - H_1 = \Delta H \quad (1)$$

where E is the energy of the system, V the volume and P the constant external pressure. The quantity $E + PV$ is defined as the enthalpy and its change in the process as ΔH. It follows from the first law of thermodynamics that, for our experimental purposes, ΔH and ΔE are nearly equal.

The measured Q, however, is comprised not only of the process contribution but also of the heats of dilution and mixing as well as secondary events, e.g., protein-protein interactions, all of which constitute thermal interferences. Hence, the possible environments for these measurements were studied so that, ultimately, the measurements could be made under those conditions which minimized interference heats. Protein-protein interactions were assumed to be negligible when lower concentrations of enzyme were present in the reaction mixture. Availability of a purified preparation of high activity was helpful in this regard.

Some media, which were otherwise appropriate, had undesirable heats of dilution when the buffered enzyme solution was mixed with the substrate, e.g., acetylcholine in water. In order to lower the dilution and mixing heat contribution, in some experiments, the enzyme and substrate were both placed in the same buffer. This, however, necessitated a set of control reactions to obtain a correction for the noncatalytic degradation of substrate at alkaline pH. The inevitable residual contributions of dilution and mixing effects were further evaluated in separate measurements and these values applied as corrections. These control measurements included buffer (containing enzyme) dilution heat and buffer protonation heat. The net reaction heat could, by convention, then be calculated from the individual reaction heats:

$$\Delta H = kQ_{\text{tot}} = Qx - (Qz - Qy) \quad (2)$$

where x is the reaction between enzyme in buffer and substrate in buffer, y the reaction between enzyme in buffer and acid in water (no substrate), z the reaction between enzyme in buffer and water (no acid or substrate), and k a calibration constant.

The validity of this correction is questionable when both the enzyme and the substrate are present in the same buffer, and the simultaneous dilution-protonation interactions of reaction 'y' do not occur (although the slower energy changes resulting from protons produced in the reaction must not, of course, be neglected). We considered alternatives to this method of data treatment: (1) To use a correction factor for the buffer protonation heat from the literature. Unfortunately, no applicable data are available in spite of the large number of thermodynamic measurements published [17] and, in any case, it is not certain that calculated values would encompass all the effects related to the local production of protons at the enzyme surface. (2) To make control measurements of acid-buffer interactions under the conditions of our experimental measurements. Data treatment using these measured values will be discussed below. (3) To eliminate the buffer altogether in the medium. Drastic as it appears, this is the least complex possible condition, and we undertook to carry out some reactions under nonbuffered conditions (fig. 8). Hydrolysis continuing under this condition results in a continuing increase in acidity to the extent that eventually the reaction kinetics are altered. To a point, this could be accommodated for a thermodynamic measurement by using high enzyme concentrations and relatively small amounts of substrate. The measured enthalpy at 0.5 μmol substrate was -1.432 kcal/mol. Higher substrate concentrations (1.5 and 3.0 μmol), however, gave lower relative heat values (fig. 8). The mean value of the three reactions was -0.931 kcal/mol. Acid production may have prevented the reaction from going to completion. However, other possibilities exist: higher substrate and/or reaction products may interact with the enzyme (homotropic allosterism or end-product

inhibition) to produce an endothermic event. We do not think that there is any enzyme inhibition by substrate itself because of the optimal levels used.

Measurements made in buffered media are summarized in table 2. The first three sets of reactions are uncorrected for the buffer protonation heats. In each instance, however, higher values were obtained for each condition when the substrate concentration was higher.

For measurements corrected by the application of previously measured heats for buffer-acid interactions, sodium phosphate buffer medium gave higher value (fig. 9) than did equivalent interactions in potassium phosphate buffer.

Measurements in which Tris-HCl buffer was used gave considerably higher values. Also in this buffer, substrate concentration had a pronounced effect upon the measured heat. At a substrate concentration of 0.1 μmol, the net exotherm, after correction for the heat of buffer protonation, was 8.0–9.2 kcal/mole – a value much greater than that measured for the hydrolysis in any other environment – buffered or unbuffered. There would appear to be an interaction of the Tris molecule intimate to the reaction which contributes very significantly to the measured heat. In a Tris environment, the ionic interplay between the substrate molecule and the protein surface is apparently much more energetic at low substrate concentrations. Until the role of the Tris molecule

Table 2

Calculated enthalpy values for acetylcholine hydrolysis under various reaction conditions

Enzyme		Substrate		ΔH (kcal/mol)
Quantity	Medium	Quantity (μmol)	Medium	
By difference between various substrate concentrations				
(1) 10000 U (1.0 mg), electric eel, affinity-purified	potassium phosphate buffer, 0.1 M, pH 7.2	6, 9, 12	same buffer	−0.830 * −1.399 *
(2) 5000 U (0.5 mg), electric ell, affinity-purified	sodium phosphate buffer, 0.1 M, pH 7.2	3, 12, 24	same buffer	−0.756 * −1.211 *
(3) 5000 U (0.5 mg), electric eel, affinity-purified	sodium phosphate buffer, 0.1 M, pH 7.2	3, 6, 30	same buffer	−1.023 * −1.066 *
By correction with a buffer-acetic acid reaction heat				
(4) 5000 U (0.5 mg), electric eel, affinity-purified	sodium phosphate buffer, 0.025 M (final), pH 7.2	3	water	−2.471
By correction with a buffer-hydrochloric acid reaction heat				
(5) 10000 U (1.0 mg), electric eel, affinity-purified	potassium phosphate buffer, 0.1 M, pH 7.2	6	same buffer	−0.107
By correction with Tris-HCl neutralization heat values				
(6) 0.2 U, electric eel, affinity-purified	Tris-HCl buffer, 0.1 M, pH 8.0	0.1 0.5	water water	−8.000 −6.480
(7) 1.0 U, electric eel (Sigma)	Tris-HCl buffer, 0.1 M, pH 8.0	0.1 0.2 0.5	water water water	−8.100 −4.300 −2.500
(8) 0.2 U, bovine erythrocyte (Sigma)	Tris-HCl buffer, 0.1 M, pH 8.0	0.1	water	−9.200

* Uncorrected for the buffer protonation heat.

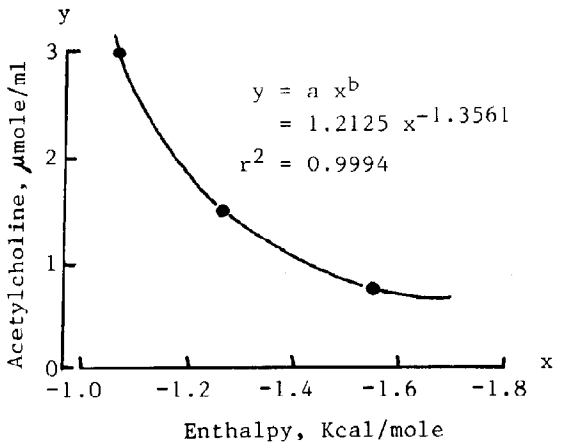

Fig. 8. Enthalpy values of acetylcholine hydrolysis in nonbuffered reaction media. Enthalpy and substrate concentration were linearly related by a negative exponent. For actual tracings, see fig. 1.

is defined for this reaction, it must be considered an inappropriate buffer for direct thermodynamic measurement of the acetylcholinesterase hydrolysis of substrate.

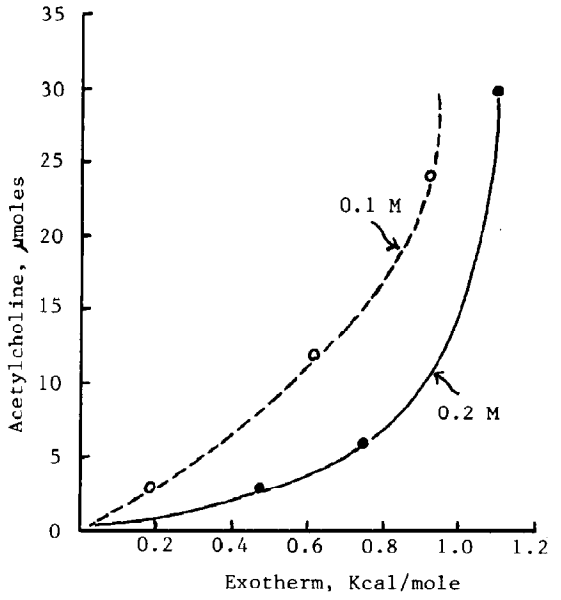

Fig. 9. Effect of sodium phosphate buffer molarity (pH 7.2) on the reaction heat between acetylcholinesterase in 1.0 ml buffer and various amounts of acetylcholine in 1.0 ml water.

Although the 'ideal' condition for the thermodynamic measurements remains elusive, for this particular system we propose that an unbuffered environment or one containing potassium phosphate buffer (corrected with a set of control reactions) appears to offer the fewest practical difficulties. The mean value for ΔH for acetylcholine hydrolysis thus was measured to be -0.107 kcal/mol under buffered conditions and -0.931 kcal/mol under nonbuffered conditions. These values are, of course, below calculated values for ester hydrolysis per se (e.g., approx. 10 kcal/mol for hydrolysis [18]).

4. Discussion

During the enzymatic hydrolysis of acetylcholine, the enzyme is first acetylated through the cleavage of the ester bond with simultaneous formation of an alcohol (choline) by the proton released from the acetylated enzyme. Acetylated enzyme then reacts with water to regain the proton and to release the acetate. Because of the instability of the acetate, however, it reacts with the hydroxyl ion in the medium to form a carboxylate anion and water.

The overall reaction is reminiscent of a base-catalyzed ester hydrolysis by acyl-oxygen fission. However, in the enzymatic reaction, the nucleophilic attack is made by a hydroxyl group on the enzyme unlike the hydroxide ion in the base-catalyzed reaction. One proton is liberated for each molecule of acetylcholine hydrolyzed.

Definition of energy changes which are concomitants of biochemical interactions is often a requisite to the understanding of those interactions. Data obtained by direct calorimetric measurements provides a basis for the discrimination between alternative models for the mechanism. Thermodynamic values have been successfully applied to a description of serine-hydrolase reactions [19–23]. Although direct measurement provides the only access to model-free thermodynamic values, other methods have often appeared sufficient to provide the necessary data. This is usually undertaken, using the van't Hoff method, by evaluating the temperature dependence of the various

equilibria involved. However, difficulties relate to the technical limitations inherent in measuring binding constants with the necessary precision at several temperatures to allow the extraction of a temperature-dependent enthalpy. For many problems, especially those related to entropy and enthalpy changes of a reaction, calorimetric measurement is the essential reference for the evaluation of all other data. Hinz [24] has elaborated the issue that "Gibbs energies are relatively insensitive to variation in the molecular details of the reaction. The degree of indeterminacy with regard to mechanism decreases, however, with increasing order of the temperature derivatives of Gibbs energy functions. Therefore, enthalpy-, entropy-, and heat capacity values form a less ambiguous basis for a molecular interpretation of the thermodynamic parameters than the Gibbs energy data alone. Determination of these quantities without model assumptions of the reaction mechanism is only possible by direct calorimetric measurements..." The values which have been calculated for various events of the acetylcholine hydrolysis sequence (cited above) are model-dependent. As such, they ultimately must be evaluated against a direct measurement. Unfortunately, for some of the events involved, it is not immediately apparent how we can experimentally separate the interaction of interest from the heat representing the sum for the entire acetylcholine hydrolysis. The measured value of the overall process does, however, provide a constraint on values that may be calculated for the individual events.

Direct measurement of thermodynamic values often presents technical problems which can interpose considerable uncertainty into the interpretation of the data in terms of mechanism. In part, the nonphysiological nature of measurement represents a difficulty in application of data to a real understanding of the biochemical system. Calorimetric measurements cannot be conducted in a genuinely physiological environment. It is the biological environment of a neuromuscular junction which constitutes the in vivo interactive setting for the membrane-bound acetylcholinesterase.

In its physiological environment, the enzyme reacts with acetylcholine which is simultaneously bound to the receptor (AChR) protein. Identity and concentrations of reactants, ions, and interfering macromolecules, are undefined. In view of this lack of definition of the true biological setting, a suggestion that one condition is physiological and another is not has no meaning. In the biochemical measurement, the best we can do is to characterize the system properly so that the contribution of elements which we impose is understood. This has not always been done in practice. The experiments described in this report, conducted under conditions which varied in media, substrate levels, and salt molarities, are an effort to expand the characterization of the reaction beyond the conventional measurement in a fully buffered system often with salts added upon an arbitrary, historical basis.

Other data relate to the contribution of Tris buffer to measurements. Generally, buffer salts' contributions to reaction thermodynamics cannot be considered negligible. Tris, and other larger buffer molecules, have been employed in calorimetric work partly because the well-quantified buffer protonation heats amplify measured events when the reaction provides a proton [14,25]. However, it is apparent from our examination of solution molarity, the hypothesized electrical character of the reaction mechanism, and an apparent role of Tris in affecting thermodynamic measurements of the acetylcholinesterase reaction, that these contributions need be understood. Although this has not been accomplished definitively, the data given here serve to describe better the contribution of Tris to the interaction heats.

It is apparent that the almost explosive reaction rate and very high specificity for the native substrate are indicative of a highly optimized system. The measured ΔH of hydrolysis reported in this paper indicating a very small evolved heat cannot in reality mean that the energetic cost of the catalysis is small. Rather, we think this value indicates that the driving force or forces are tightly coupled to the events of the catalysis. That is to say, we have a function optimal in its rapidity, substrate specificity, and in energetic efficiency. Our measurements indicate that although a large energy exchange must be involved in this catalysis, endothermic events are so perfectly coupled to exothermic events that there is little 'waste' heat to be measured.

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