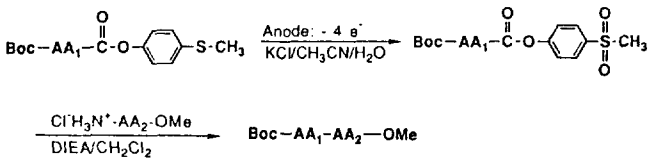

Scheme 1.

Table 2. The Results of Dipeptide Synthesis

Dipeptide	Overall Yield (%)	MP (℃)	Lit mp. (℃)	Ref
Boc-Phe-Phe-OMe	53	114-115	114-115	15[a]
Boc-Phe-Gly-OMe	48	90	91	15[b]
Boc-Phe-Val-OMe	50	119-120	120-122	15[c]
Boc-Ala-Gly-OMe	41	oil	oil	15[d]
Boc-Leu-Gly-OMe	52	132-133	131-132	15[e]
Boc-Leu-Val-OMe	40	146-147	143-147	15[f]
Cbz-Phe-Leu-OMe	55	109-110	109-109.5	15[g]
Boc-Gly-Gly-OMe	41	oil	–	

comparison to these two electrogenerated halogen species, HOCl has a much stronger oxidizing ability (E_o = 1.484 V) than Br_2(aq) (E_o = 1.066 V).[13] Moreover, it can act as a direct oxygen source.[14] So it becomes clear that the electrogenerated HOCl can oxidize Boc-Phe-OMTP more effectively. With these results we have prepared various dipeptides from Boc (or Cbz)-amino acid MTP esters (Scheme 1).

After the same electrochemical oxidation, the crude products were treated with aq. $NaHSO_3$ to quench any remaining active halogen species, and saturated with KCl. The organic layer was separated, evaporated to an oily residue, which was crystallized in acetonitrile/water. Then, 3 equiv. of amino acid methyl esters and 3 equiv. of diisopropylethylamine (DIEA) were added to the MSO_2P ester in methylene chloride. After stirring overnight at room temperature and the usual work-up gave dipeptides in 40-55% overall yields. The results are summarized in Table 2.

In conclusion, the direct electrooxidation of amino acid MTP ester has only yielded the corresponding sulfoxy ester. But when KCl was used as a mediator, the electrochemical oxidation of MTP ester gave the corresponding sulfonyl ester. Then, the amino acid MSO_2P esters were easily coupled with another amino acid derivatives, yielding dipeptides effectively. We are now focusing on the optimization of the electrooxidation conditions to improve the yields.

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Temperature-Dependence of Mouse Brain Membrane-Bound Acetylcholinesterase Systems: Manifestation of $\Delta H^{\neq}$-$\Delta S^{\neq}$ Compensation

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Acetylcholinesterase (AChE, EC 3.1.1.7) from a variety of sources has been found in association with membranes and in many cases tightly embedded in the membrane[1-3]. However, the exact relationships of the enzyme kinetic properties with this association and moreover the effect of temperature-dependent phase transition of the membrane-lipid on the

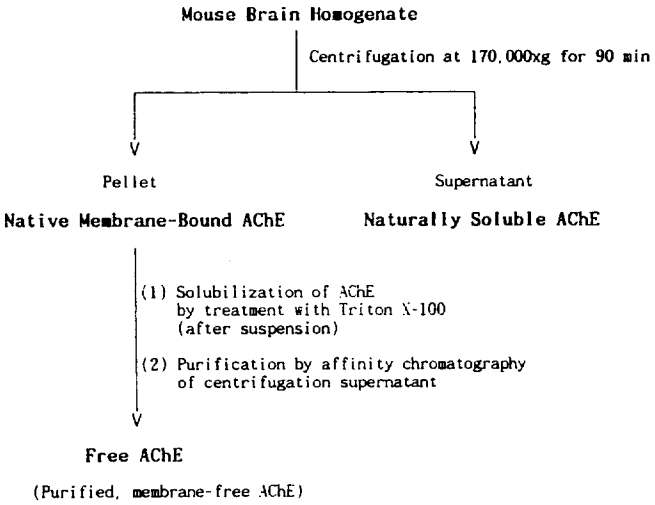

Figure 1. Schematic outline for the preparation of "native membrane-bound" and "free" AChE. For more detailed flow sheet, see Reference 5.

thermodynamics concerning the enzyme activity has not been fully clarified. In the present work we investigated the effect of a compensation law, activation enthalpy-entropy compensation, involved in the mouse-brain AChE kinetics which is accompanied with the total phase change of the membrane-lipid.

Experimental

The solubilization and purification of AChE and the preparation of native membrane-bound (crude) AChE from the mouse brain homogenate were carried out as described previously[4] (see the flow sheet in Figure 1 for the schematic outline of the preparation of the AChE species.). Liposomes (neutral) and reconstituted AChE in liposomes (100% dipalmitoylphosphatidyl choline liposome-AChE complexes) were prepared as described previously[5]. Briefly, after removal of chloroform from the chloroform solution of the phospholipid, 4 m*l* of 0.05 M phosphate buffer, pH 7.4 was added together with several glass balls, and then the solution was flushed with N_2 and agitated for 10 min to get the phospholipid vesiculated to form liposomes. For the formation of the reconstituted enzyme, a 0.2 m*l* portion of the liposomes was mixed with 0.8 m*l* of the phosphate buffer and 0.1 m*l* of 5,5'-dithiobis-(2-nitrobenzoate), and then the purified (free) enzyme (5 μ*l*) was added, thoroughly mixed and incubated at 25℃ for 1 hour.

For the van't Hoff plot for the each AChE species, *i.e.*, free AChE, native membrane-bound AChE, reconstituted AChE, the AChE activity (v) of the AChE species was determined in 0.1 M phosphate buffer, pH 7.4 by the colorimetric method of Ellman *et al.*[13] with acetylcholine iodide as substrate over a wide range of the substrate concentration ([S]). The maximum enzyme activity (V_{max}) was then obtained from the Lineweaver-Burk plot (1/v *vs.* 1/[S] plot).

In the van't Hoff plot, $\log(V_{max}/T)$ was plotted against the reciprocal of the absolute temperature (T). The temperature range of the plot was between 5 ℃ and 50 ℃, over which the enzyme species were stable.

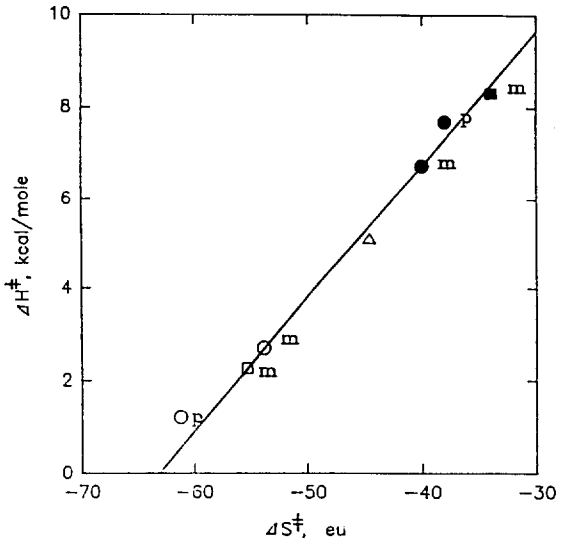

Figure 2. Compensation plot for the activity of: the mouse-brain AChE species, *i.e.*, free ($\triangle_m$); reconstituted, above ($\square_m$) and below ($\blacksquare_m$) transition temperature; natural-membrane-bound, above ($\bigcirc_m$) and below ($\bullet_m$) transition temperature; pig-brain natural-membrane-bound AChE, above ($\bigcirc_p$) and below ($\bullet_p$) transition temperature. Data were obtained in 0.1 M sodium phosphate buffer. pH 7.4 (for mouse-brain enzyme) and pH 7.9 (for pig-brain membrane-bound enzyme) by the colorimetric method of Ellman *et al.* (1961) with acetylcholine iodide as substrate.

Results and Discussions

From the slope of the van't Hoff plot and the absolute reaction rate theory for the AChE activity in the various environments (*i.e.*, free, reconstituted, and native membrane-bound enzyme species, respectively), the values of activation enthalpy (ΔH^*) and activation entropy (ΔS^*) respectively were obtained for the construction of ΔH^*-ΔS^* compensation plots. In the van't Hoff plots a single straight line was obtained for the purified free enzyme, whereas both the graphs for the reconstitued enzyme and the membrane-bound enzyme had a discontinuity of slope at 28.2℃ and 26.4℃ respectively. The existence of two independent straight lines meeting at an angle indicates that there are two different transition states involved for the process under consideration and there is a change from one value of activation energy to another at the transition temperature. A number of enzymes that are complexed with membrane material exhibit such discontinuities, and in many cases these have been correlated with phase changes in the bound lipids[6,7].

Although the possibility of discontinuities arising from other causes has also been cited previously for other membrane enzymes[8,9], in the present case of the mouse-brain membrane AChE no indication of such complications were recognized: K_m values remained the same over the whole temperature range investigated, and the purified enzyme assayed in the absence of Triton X-100 exhibited a linear van't Hoff plot. Discontinuities around the phase transition temperature of membrane lipid in van't Hoff or Arrhenius plots of membrane bound enzymes have widely been interpreted to be a reflection of a lipid phase transition and therefore, to indicate a lipid dependence of the enzyme kinetics[10,11].

When $\Delta H^{\neq}$ values of both before and after lipid-phase transition for the native membrane-bound and reconstituted enzyme species were plotted against $\Delta S^{\neq}$ values, a linear relationship ($\Delta H^{\neq}=\alpha+Tc\cdot\Delta S^{\neq}$) was observed. The ($\Delta H^{\neq}$, $\Delta S^{\neq}$) points for the free-enzyme species and those for the pig-brain membrane-bound AChE[12] fell on the same straight line with a correlation coefficient of 0.998 (see Figure 2). This observation indicates the existence of an enthalpy-entropy compensation effect in the enzyme mechanism. The slope of the line on the compensation plot of the activation enthalpy versus activation entropy [the so-called "isoequilibrium", "isokinetic" or "compensation" temperature, *Tc* (see Lumary & Rajender[14])] was found to be 300 °K. This observed compensation temperature lies well within the range of 260-315 °K of Likhtenshtein's compilation[15], Vaslow-Doherty pattern of 250-320 °K[16] and the range of 250-315 °K of Lumry-Rajender compilation for small solutes in water[14].

Consequently the present data show that the precision of the enthalpy-entropy compensation phenomena of the AChE activity in water solutions is not affected by the associated lipids and the lipid states. Our results do not support the previous argument made by Kumamoto *et al.*[17] that the enthalpy-entropy compensation might not be quite exact if the total phase change is dominated by non-active sites, as might be the case where the major reorganizations involved in the phase change take place in the lipid portion of a lipoprotein, and that in such case two straight lines with different slopes and a non-intersecting discontinuity would be obtained on the Arrhenius plot.

The importance of non-active-site regions of enzymes as chemodynamical machines capable of coupling the active-site reactivity with the molecular events of the enviroment has become apparent[18], and parallel enthalpy and entropy changes which compensate each other to produce minor changes in the free energy changes of the processes has been proposed at the root of the coupling of chemical processes to the properties of the environment[14].

Likhtenshtein[15] proposed that enthalpy-entropy compensation plays an essential role in enzymic mechanism and he associated it with both protein conformation and solvent shell. If thermodynamic compensation provides a potential means for producing large changes in protein geometry with small expenditure of work as stated by Lumry and Rajender[14], then compensation processes will offer attractive possbilities in the search for explanations of membrane-enzyme functions.

Thus present data support the general idea that most examples of compensation behavior for processes in water solution are due to the dominant role, *i.e.*, a single unique characteristic of liquid water as a solvent regardless of solutes and solute processes studied[14]. The significance of the enthalpy-entropy compensation for the enzymic catalysis would be found in the "rack" mechanisms proposed by Eyring *et al.*[19].

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