Communications to the Editor

[Chem. Pharm. Bull. 34(6)2684—2686(1986)]

THE PHOTOAFFINITY LABELING WITH A TETRODOTOXIN DERIVATIVE OF A SODIUM CHANNEL PROTEIN FROM THE ELECTRIC EEL[1)]

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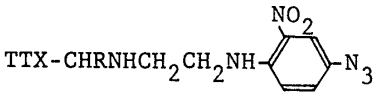
The 250 kDa polypeptide of the electric eel sodium channel was specifically labeled with a photoactivatable tetrodotoxin derivative.

KEYWORDS —— photoaffinity labeling; sodium channel; electric eel; tetrodotoxin; gel electrophoresis:

Tetrodotoxin (TTX), a structurally unique heterocyclic guanidine,[2)] is one of the specific neurotoxins which bind to the sodium channel to block its ion transport. TTX provides a powerful tool to explore the sodium channel molecule biochemically,[3)] as well as pharmacologically or electrophysiologically.[4)] For example, sodium channel protein from electric eels was successfully solubilized and purified[5)] by [^{3}H]-TTX binding to the channel molecule as a marker of its biological activity. The recent elucidation of the complete amino acid sequence of eel sodium channel by us[6)] inaugurates a new phase of research on the molecular properties of ion channels in which the molecular architecture of their functional components is revealed at ever finer resolution. As the first approach along this line of research, we report here a successful photoaffinity labeling of eel sodium channel protein (250 kDa) by a [^{3}H]-TTX derivative.

The TTX derivative, the 2-nitro-4-azidophenyl ethylenediamine conjugate of tetrodotoxin (Nap-en-TTX) 1, was synthesized by a modification of Lazdunski's method[7)] to give a 3~6-fold increase in overall yield (6%). Two milligrams of citrate-free TTX (Sankyo Chemical Co.) were oxidized by the described procedure,[8)] and the Nap-en adduct of the oxidized TTX was reduced with $NaBH_3CN$. The Nap-en-TTX fraction, separated by cellulose TLC,[7)] was finally purified by an ODS reverse-phase HPLC column (Nucleosil, 0.4 x 25 cm) with 0.1% TFA - CH_3CN elution. A tritiated compound of 1 was similarly synthesized using $NaB[^3H]_3CN$ (10 mCi, 3.6 umol: Amersham) instead of $NaBH_3CN$, and the product obtained has a reasonable specific radioactivity (2.5 Ci/mmol).



R=H 1, R=[^{3}H] 2

In the dark [^{3}H]-Nap-en-TTX 2 reversibly binds to the membrane fragments prepared from electric eel electroplax in a saturable manner. A Scatchard plot of the specific binding showed K_d = 14 nM and B_{max} = 2.8 pmol/mg of protein. These values are comparable to those of [^{3}H]-TTX[9)] and the derivative meets the first requirement for a probe to identify the TTX binding site of the sodium channel.

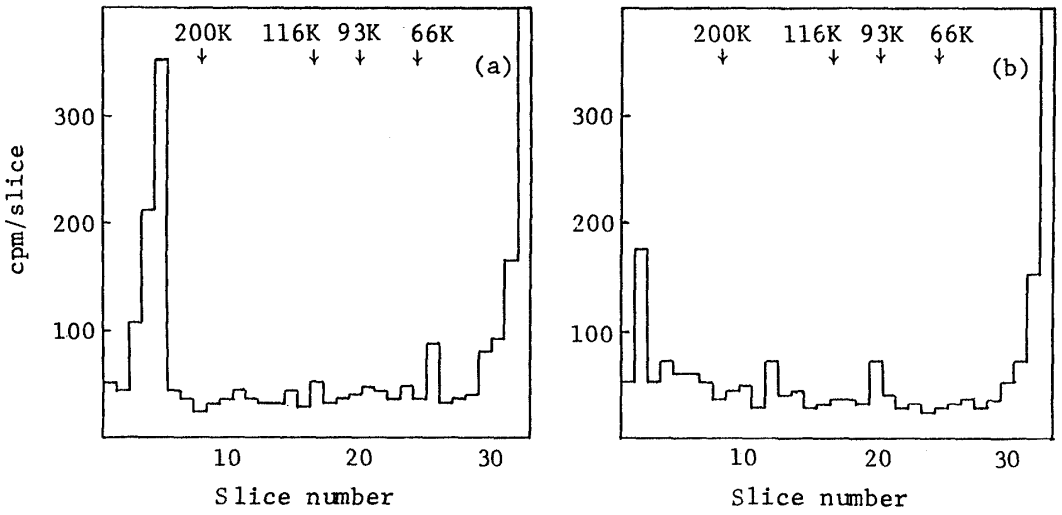


Fig.1. Distribution of Radioactivity Following SDS Gel Electrophoresis of Eel Sodium Channel Photolyzed with 2 in the Absence (a) and Presence (b) of 10 μM TTX

When 0.5 μM of 2 was incubated with the solubilized and DEAE-Sephadex-purified eel sodium channel (150 pmol of Nap-en-TTX sites/mg of protein, 0.6 μM) for 30 min at 0°C, a calculated 92% of the channel protein formed a reversible complex with 2. This mixture was irradiated with 100 flashes of a Xenon lamp (Panasonic PE-3000) covered with a Pyrex filter. Analysis of the irradiated sample with a disc SDS-PAGE (6% gel) indicated that the 250 kDa polypeptide was labeled mostly in a covalent manner (Fig. 1a). The labeling was markedly suppressed in the presence of TTX (10 μM) during irradiation (Fig. 1b). These results clearly show that 2 specifically labels the 250 kDa polypeptide.

This is the first demonstration that the large polypeptide of the eel sodium channel can be photolabeled with a tetrodotoxin derivative. As the complete amino acid sequence of the 250 kDa polypeptide is now available,[6] this procedure is a highly promising tool for revealing the TTX binding site on the primary structure by determining the photolabeled sites with 2. Peptide mapping and identification of the labeled sites are now in progress.

ACKNOWLEDGEMENT This work was supported in part by Grants in Aid for Scientific Research (60470143) and for Special Research on Molecular Mechanism of Bioelectrical Response (60215003) from the Ministry of Education, Science and Culture, Japan, and grants from Suzuken Memorial Foundation and the Japan Society for Promotion of Science.

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(Received April 25, 1986)