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Interaction of modified neurotoxins from *Naja nigricollis* with the nicotinic acetylcholine receptor from *Torpedo marmorata*

A Raman spectroscopy study

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Two derivatives of α-toxin from *Naja nigricollis* venom were used in order to study, by resonance Raman spectroscopy, its interaction with the nicotinic acetylcholine (AcCho) receptor from membranes of *Torpedo marmorata* electrocytes. The two modified toxins carry either an NO_2 group bound to Tyr^{25} or a nitrophenylthioether (NPS) bound to Trp^{29}. The comparison of the spectra of the free and bound derivatized toxins indicates that the environment of Tyr^{25} is not perturbed upon binding to the AcCho receptor, but the surroundings of NPS bound to Trp^{29} are changed. This result indicates that Tyr^{25} is not involved in binding, while Trp^{29} of the α-toxin may be in contact with the AcCho receptor. Examination of the spectrum of the AcCho receptor membrane after binding of the NPS-Trp toxin discloses some modifications of the vibrations of the tryptophan and cysteine disulfide bridge of the receptor. These residues are possibly involved in toxin binding.

Acetylcholine receptor; Snake neurotoxin; Protein interaction; Raman spectroscopy

1. INTRODUCTION

The acetylcholine nicotinic receptor is a major component of the postsynaptic membrane of the electromotor synapse and the neuromuscular junction. This allosteric membrane protein of 300 kDa possesses a pentameric 'heterologous' organisation $\alpha_2\beta\gamma\delta$ where each α-subunit carried a binding site of neurotoxins. Its initial identification from fish electric organ [1] and the subsequent characterisation of its functional architecture [2–4] has received considerable help from the utilisation of snake venom α-toxins [5–7] which behave as competitive antagonists of the physiological response and bind in a highly selective and slowly reversible manner to the acetylcholine binding site. The AcCho receptor–α-toxin complex displays a very low dissociation constant (K_d=2×10^{-11} M) suggesting that multiple side-chain interactions take place in toxin-receptor binding [8]. An important issue in the understanding at the molecular level of the mode of action of the neurotoxins is the identification of the side-chains involved in the binding sites of both molecules. Studies of the interaction of the toxin α from *N. nigricollis* using EPR [9], fluorescence spectroscopy [10], chemical modifications [11] and photolabelling experiments [12] have been reported. Amino acids which belong to the binding site for cholinergic ligands on the α-subunit have been identified by means of affinity reagents which covalently bind to the acetylcholine binding site [13,14] or by binding of the α- neurotoxin to proteolytic fragments of the α-subunit [15–17]. These studies disclosed the importance of several residues of the α-subunit: Trp^{149}, Tyr^{190} and $Cys^{192-193}$.

Raman spectroscopy [18,19] is a particularly well-suited technique to investigate the interaction of proteins because many vibrations are environment-sensitive. However, to analyse the Raman spectra of the receptor–toxin complex a method that allows the 2 molecules to be distinguished is required. For this purpose, we have used two derivatives of toxin α from *N. nigricollis*, on which 2 kinds of chromophores are specifically bound. One derivative had incorporated an NO_2 group bound to the conserved Tyr^{25}, and the other carries a nitrophenylthioether group (NPS) bound to the invariant Trp^{29} [7]. Resonance Raman spectroscopy makes possible the enhancement of the vibrations of a single group by irradiating the sample with an excitation wavelength close to an electronic absorption band of the added chromophores. For the tyrosyl and tryptophanyl derivatives an excitation with argon ion laser at 458 nm gives rise, respectively, to resonance and pre-resonance conditions, and these chemical groups can be readily identified by their vibrational characteristics.

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2. MATERIALS AND METHODS

Postsynaptic membranes were prepared according to [20,21] from *Torpedo marmorata* electric organ in the presence of protease inhibitors. The postsynaptic membranes were washed, concentrated by centrifugation and were used immediately after preparation. The concentration of toxin binding sites of the receptor was measured with tritiated toxin α as described by [22]. The protein concentration was typically 10–20 mg/ml, and the specific activity 2.5–3.0 μmol of toxin sites/g of protein (0.4–0.5 g of active receptor/g protein).

The two derivatives [25-C_3-nitrophenol]toxin α and [29-C_2-[(nitrophenyl)thioether]indole]toxin α were prepared as described in [7] and [11], respectively. The freeze-dried modified toxins were dissolved in 10^{-2} M Tris buffer, pH 7.4, to obtain a concentration of 20 mg/ml.

The binding to the AcCho receptor was performed by incubating (12 h at 4°C) membranes with modified toxins in molar excess compared to agonist sites. Excess of toxin was removed by 3 centrifugations (5000 × *g*, 5 min) and dilution steps; the toxin-saturated membranes were used immediately. Even in the case of modified toxins, the dissociation constants were very low; thus at equilibrium, the quantity of free toxin was not significant. The pre-resonance spectra were those of bound, modified toxins.

The Raman experiments have been carried out as described in [7].

3. RESULTS AND DISCUSSION

3.1. *Toxin modified at Tyr^{25}*

In this first interaction experiment, we have used toxin α whose conserved tyrosine is modified by addition of an NO_2 group. We recorded the Raman spectra of nitrotyrosyl toxin free in solution and bound to the postsynaptic membrane at pH 7.5 with 458 nm excitation. The resonance vibrations of the nitrophenol chromophore are the conspicuous features in both spectra (Fig. 1). The spectrum of the nitrotyrosyl toxin free in solution (Fig. 1a) discloses a very strong peak located at 1341 cm^{-1}. This $\nu_s(NO_2)$ vibration of the nitrophenol chromophore, the intensity of which is the largest, comes from resonantly enhanced symmetric stretch, and is sensitive when a change in phenol state occurs, and the NO_2 group can be used as a probe of tyrosine environment [23]. The minor peaks located at 776, 830, 898 and 1270 cm^{-1}, whose intensity is lower, are assigned to the chromophore. The 830 cm^{-1} phenol ring vibration has also been shown to be sensitive to the phenol state [24] and provides another probe for the environment of the tyrosine.

The main peak located at 1341–1342 cm^{-1} appears in both spectra (Fig. 1) with the same frequency (a difference of 1 cm^{-1} is within the spectral resolution). The frequency, as well as the intensity ratio of the other peaks assigned to the chromophore, particularly that located at 830 cm^{-1} characteristic of the hydrogen-bonded phenol of Tyr, clearly remain unchanged when the toxin is bound (Fig. 1b). The minor peaks of the nitrotyrosyl toxin are hidden or deformed by those of the membrane. In the spectrum of the toxin–AcCho receptor complex many vibrations of the membrane are present but not resolved because of the presence of strong peaks from the chromophore (the 1669 and 1445 cm^{-1} components are respectively due to amide I and $\delta(CH_2)$ vibrations of the postsynaptic membrane). The invariance of Raman vibrational signals from the nitrophenol-modified tyrosine shows that the hydrogen bond in which the tyrosinic phenol participates, as shown previously, is not modified. This indicates that the environment of Tyr^{25} is not disturbed by the binding of the toxin to the receptor.

The vibrations of the nitrophenol group were studied under resonance conditions [23] in the case of egg-white lysozyme. It is assumed that the frequencies of 1340 and 1330 cm^{-1} correspond, respectively, to a hydrophilic and a hydrophobic environment with a strong H-bond involving the phenol. We have observed a value of 1340 cm^{-1} for the NO_2-Tyr of the toxin, which is assumed to be involved in a strong H bond on the basis of the tyrosine vibrational component located at 830 cm^{-1}. Moreover, the toxin is not denatured as indicated by the high remaining toxicity and by its unchanged affinity towards the $M_{\alpha 1}$ antibody [7]. This value of the $\nu(NO_2)$ frequency could be related to the fact that Tyr^{25} of the homologous erabutoxin does not appear completely buried, upon examination of the X-ray structure, albeit inaccessible [25]. But, if Tyr^{25} was involved in direct binding to a side-chain of the receptor by means of a phenol H-bond, this frequency would change. Thus, the presence of a H-bond involving Tyr^{25}, and the absence of modifications for 2 environment-sensitive vibrations (830 and 1341 cm^{-1}), together with the absence of substantial change in toxin affinity binding after nitration, indicate that Tyr^{25} is not directly involved in the complex formation between toxin and AcCho receptor. Rather it plays a structural role, by stabilizing the β-sheet structure of the α-toxin.

3.2. *Toxin modified at Trp^{29}*

The absorbance maximum of NPS-Trp^{29} toxin is located at 366 nm, and the chromophore is in pre-resonance with excitation at 458 nm. The very strong peak from $\nu_s(NO_2)$ vibration in pre-resonance conditions located at 1342 cm^{-1} in free NPS-toxin spectrum (Fig. 2a) is shifted to 1347 cm^{-1} when the toxin is bound (Fig. 2b). This shift could be due to the addition of the 2 signals located at 1342 cm^{-1} in the free toxin spectrum and 1352 cm^{-1} in the free AcCho receptor membrane spectrum (Fig. 2c) and not directly due to the interaction. The intensity of $\nu(NO_2)$ must only be compared to another one from the bound chromophore, because the concentration of Trp-NPS modified toxin was lower when the toxin was bound. The intensity of this vibration decreases in comparison with another chromophore vibration: the stretching vibration $\nu(\phi\text{-N})$ which is very weak in the case of the free toxin (Fig. 2a) appears enhanced at 1228 cm^{-1} after binding (Fig. 2b). We readily assigned the peak located at 1228 cm^{-1} to the chromophore bound to the toxin since no peak is seen in the spectrum of the free receptor membrane (Fig. 2c). An increase in intensity of this vibration has been observed [26] in the

case of arsanylazotyrosine when the nitrogen is involved in the formation of an intermolecular H-bond. This increase of intensity of the $\nu(\phi$-N) vibration with respect to that of the $\nu_s(NO_2)$ could therefore indicate a change in the polarity of the environment of the chromophore NPS added to the Trp^{29} of the α-toxin. This spectral modification may reveal a contact or close proximity between the receptor surface and NPS covalently bound to Trp. We do not see directly the Trp^{29} to which the NPS is bound. However, the presence of the bulky NPS group likely gives rise to a steric hindrance, and the proximity of the NPS chromophore and AcCho receptor after binding of the modified neurotoxin α strongly indicates that Trp^{29} is in contact with the receptor in the native toxin bound to the acetylcholine receptor. The decrease of affinity of NPS–Trp toxin [11], which does not arise from an alteration of secondary structure [7], is consistent with this view.

3.3. *The AcCho receptor membrane*

Contrary to the case of nitrotyrosyl toxin bound to the receptor-membrane (Fig. 1b), the vibrations of the membrane are not hidden by those of the NPS and are distinguishable in the spectrum of Trp-NPS toxin–AcCho receptor complex (Fig. 2b), albeit with a lower signal/noise ratio; it can be compared to the spectrum of the unbound receptor-membrane (Fig. 2c). Several amino-acid vibrations are sensitive to the conformation and to the environment of the vibrating groups [24,26–29]. Since the low signal/noise ratio does not permit an extensive interpretation, we will focus only the discussion to the two most obvious spectral changes.

In the spectrum of the free Trp-NPS toxin (Fig. 2a), the aromatic ring vibrations from Trp (1151, 1569 and 1617 cm^{-1}) are weak and not visible in the spectrum of toxin–AcChR complex (Fig. 2b). The most conspicuous signal from Trp in the spectrum of the native receptor membrane is located at 1583 cm^{-1}. The intensity of this vibration dramatically decreases when the toxin is bound to the receptor. A qualitative comparison can be made using the aliphatic vibrations as a standard [27], located at 1444 cm^{-1} in the spectra of Fig. 2b and c. This vibrational band, which is a mixture of the $\delta(CH_2)$ and $\delta(CH_3)$ deformations from aliphatic side-chains and from lipids, is expected to remain unchanged upon binding of the toxin since the lipids are not concerned by the binding, and the receptor remains in the resting state [30], i.e. does not change conformation. Therefore, the change of the vibration located at 1583 cm^{-1} may reflect a difference in Trp environment upon complex formation: these Trp residues may belong to the receptor and/or to the toxin. It has been reported [29] that the frequency of this ν(C=C) vibration of the indole ring of Trp in proteins could be shifted when modifications in the polarity of environment occur. We observed in the case of the enzyme acetylcholinesterase changes of the Trp vibration located at 1580 cm^{-1} upon different environments [31]. The change in indole vibrations may be due to the involvement of Trp residues in the site of binding of toxin α, but it is not possible to determine which of the Trp are involved. Since several side-chain interactions are involved in neurotoxin binding [8,11] we cannot extend this interpretation to the agonist binding site. Indeed, the binding site of neurotoxins could be larger and overlap the binding site of acetylcholine. Our observations could be related to the mapping experiments of the active site of the receptor. A radioactive photo-affinity label, which acts as a competitive antagonist of the cholinergic receptor [13], has been covalently bound to 4 residues of the α-subunit (Trp^{149}, Tyr^{190}, Cys^{192}, Cys^{193}). Moreover, the covalent binding of this label is prevented by the presence of a snake venom toxin. These residues are conserved in the known sequences of the AcCho receptor α-subunits from different species [32] and lie in a domain of the α-subunit primary structure which faces the synaptic cleft, according to the models of tertiary structure predicted from the sequence analysis [33,34]. Thus, these 4 amino acids are likely involved in the acetylcholine binding site. Possibly, the Trp^{149} is that (or one of those) implicated in the vibrational change that we observed.

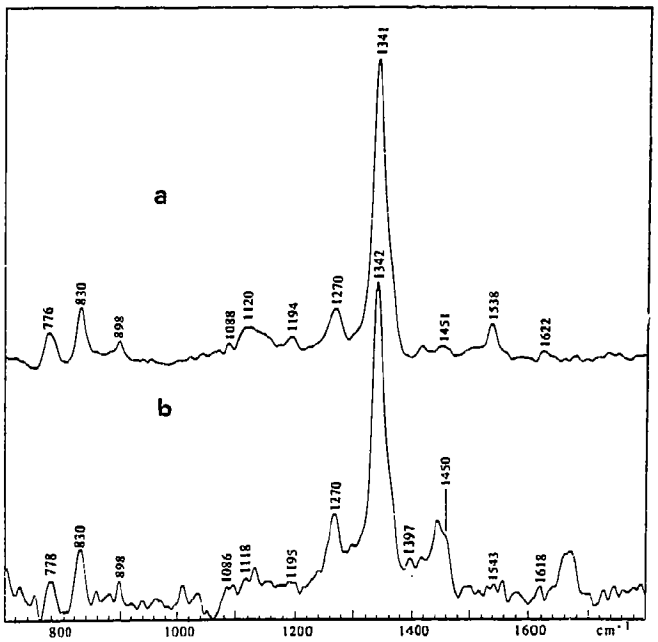

Fig. 1. Raman spectra of nitrotyrosyl toxin (a) free in Tris buffer, pH 7.5., and (b) bound to postsynaptic membrane in the same buffer conditions. λ_0=458 nm; T=10°C; P=50 mW; spectral slits, 4 cm^{-1}. All the peaks are due to the nitrophenol chromophore. Frequencies in cm^{-1} and assignments of the main vibrations (ν is stretching vibration): 776–778, para-disub. benz. 830: ring vib. 898: ring vib. 1270: $\nu(NO_2)+\nu$(C–OH). 1341–1342: $\nu(NO_2)$ sym. 1538–1543: $\nu(NO_2)$ asym.

Conspicuous modifications are visible in the region of disulfide vibrations in the receptor spectrum upon toxin binding. As the ν(S–S) stretching vibrations do not appear in Trp–NPS toxin spectrum (Fig. 2a), we

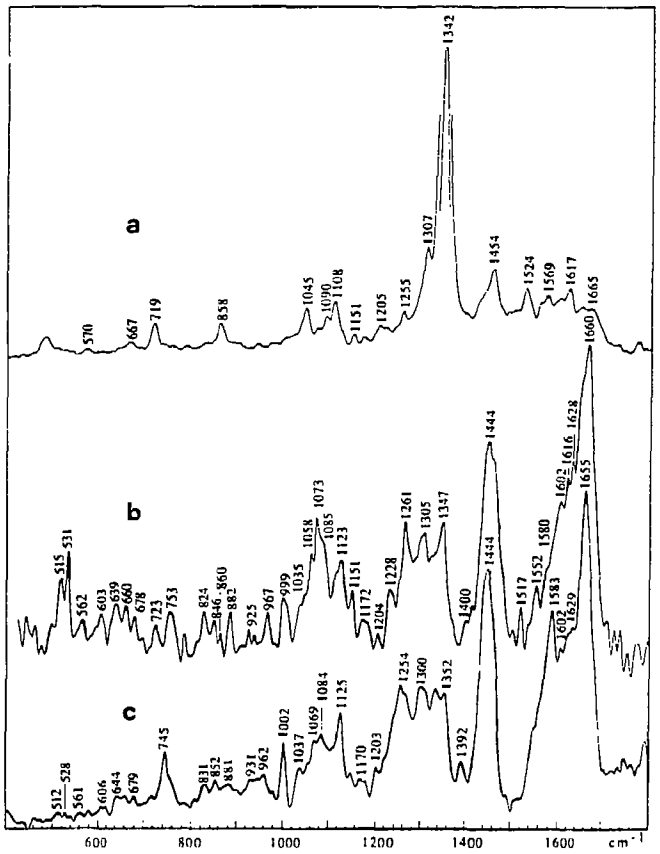

Fig. 2. Raman spectra of Trp-NPS modified toxin (a) free in buffer and (b) bound to postsynaptic membrane, compared with (c) native membrane in the same conditions. λ_0=458 nm; T=10°C; spectral slits, 4 cm^{-1}; P=50 mW for (a); P=80 mW for (b) and (c). Frequencies (cm^{-1}) and assignments for NPS-Trp modified free (a) and bound (b) toxin (+mb, peaks or bands with contribution from the membrane. ϕ is the phenyl ring. ν is stretching vibration, δ is deformation vibration: 719–723: ν(C–C) nitrophenyl. 858–860: nitrophenyl ring. 1045–1058: orthophenyl ring vib. 1108–1113: ν(C–C) nitrophenyl. 1151–1151 (+mb): ν(C–C)+ν(ϕ–N) indole vib. 1220–1228: ν(ϕ–N) 1307–1305 (+mb): nitrophenyl ring vib. 1342–1347 (+mb): ν(NO$_2$) sym. 1454: δ(CH$_2$)+δ(CH$_3$). 1524–1517: ν(NO$_2$) asym. 1552–1617: indole ring vib.

Assignments for Raman spectra of native membrane (c) and membrane with NPS–toxin (b) (+tox, contribution from toxin): 512–531: ν(S–S) Cys. 745–753: Trp vib. 881–882: ν(C–C)+δ(N–H) Trp. 999–1002: Phe ring vib. 1123–1125: ν(C–C)+ν(C–N) side-chains. 1254–1261: amide III. 1300 (+tox): δ(CH$_2$) side-chains. 1352: pyrrole ring vib. 1444: δ(CH$_2$)+δ(CH$_3$)side-ch.+lip. 1580–1583: Trp+Phe. 1655–1660 (+tox): amide I.

readily assigned the peaks present at 515 and 531 cm^{-1} in the spectrum of the complex (Fig. 2b) to disulfide bridges in postsynaptic membrane. The same vibrations appear at 512 and 528 cm^{-1} respectively, and are very weak when the toxin is not bound to AcCho receptor (Fig. 2c). The low intensity of the ν(S–S) vibrations preclude a description of disulfide geometry in the native membrane. However, in the presence of the modified toxin, an important increase of intensity for the 531 cm^{-1} component was noticed, which possibly indicates a change in the conformation of the dihedral angle χ(SS–CC) for some AcChr disulfide bridges [35,36]. Interestingly, this increase does not seem directly related to the number of disulfide bridges present in the protein. Moreover, the ν(S–S) vibration is not enhanced by electronic resonance; the intensity at 528 cm^{-1} is very weak in the spectrum of the antive membrane (Fig. 2c) with 458 nm excitation, and no peak is visible for the free modified toxin (Fig. 2a). This increase in intensity may hypothetically correspond to the phenomenon described by [37]. These authors have shown the possibility of an 'energy transfer by vibrational resonance' for these two vibration modes, the frequencies of which are very close. If such a vibrational coupling gives rise to the enhancement of the vibration at 531 cm^{-1}, the groups in contact and vibrating at close frequency could be a disulfide in AcCho receptor and the Trp-NPS in modified toxin. In that case, these side-chains would be involved in the binding of both molecules. A vibrational indole ring mode exists at a frequency close to that of the ν(S–S). Indeed, a Trp vibration has been observed, the intensity of which is weak, near 544 cm^{-1} in the cases of the lysozyme spectrum [38] and retinal opsin membrane spectrum [39].

Several experiments have shown the presence of a disulfide formed by Cys^{192} and Cys^{193} of the α-subunit within or very close to the binding site [13,14,40]. One may note that this disulfide bridge involves two adjacent cysteines, and does not appear critical for the overall tertiary structure and thus may have another important function. It has been suggested [13] that the electronegative subsite of AcCho receptor α-subunit could be formed by one or several of these 4 residues respectively by means of the phenolic OH of Tyr^{190}, the sulfur atoms of Cys$^{192-193}$ and the nitrogen of the Trp^{149} indole ring. These side-chains could have an importance in the binding interactions and/or in the architecture of the agonist site. The modification of the side-chains environment of AcCho receptor, particularly the cysteines, as a consequence of the binding of the α-toxin requires further investigations.

REFERENCES

[1] Changeux, J.P., Kasai, M., Huchet, M. and Meunier, J.C. (1970) CR Acad. Sci. (Paris) 270D, 2864–2867.
[2] Changeux, J.P. (1989) Fidia Research Foundation: Rita Levi-Montalcini, Neuroscience Award Lecture.
[3] Hucho, F. (1986) Eur. J. Biochem. 158, 211–226.
[4] Anholt, R., Lindstrom, J. and Montal, M. (1985) in: The Enzymes of Biological Membranes, vol. 3 (Martonosi, A.N. ed.) Plenum Press, New York, pp. 335–401.
[5] Lee, C.Y. and Chang, C.C. (1966) Mem. Inst. Butantan Sao Paulo 33, 555–572.
[6] Lee, C.Y. (1973) Proc. Int. Congr. Pharmacol. 5th, San Francisco, 2, 210–232.
[7] Négrerie, M., Grôf, P., Bouet, F., Ménez, A. and Aslanian, D. (1990) Biochemistry 29, 8258–8265.
[8] Weber, M. and Changeux, J.P. (1974) Mol. Pharmacol. 10, 1–14.
[9] Rousselet, A., Faure, G., Boulain, J.C. and Ménez, A. (1984) Eur. J. Biochem. 140, 31–37.

[10] Tsetlin, V.I., Karlsson, E., Utkin, Y.U., Pluzhnikov, K.A., Arseniev, A.S., Surin, A.M., Kondakov, V.V., Bistrov, V.F., Ivanov, V.T. and Ovchinikov, Y.A. (1982) Toxicon 20, 83–93.
[11] Faure, G., Boulain, J.C., Bouet, F., Montenay-Garestier, T., Fromageot, P. and Ménez, A. (1983) Biochemistry 22, 2068–2076.
[12] Chatremet, B., Trémeau, O., Bontems, F., Goeldner, M., Hirth, C. and Ménez, A. (1990) Proc. Natl. Acad. Sci. USA 87, 3378–3382.
[13] Dennis, M., Giraudat, J., Kotzyba-Hibert, F., Goeldner, M., Hirth, C., Chang, J.Y., Lazure, C., Chrétien, M. and Changeux, J.P. (1988) Biochemistry 27, 2346–2357.
[14] Kao, P., Dwork, A., Kaldanu, R., Silver, M., Wideman, J., Stein, S. and Karlin, A. (1984) J. Biol. Chem. 259, 11662–11665.
[15] Wilson, P.T., Lentz, T.L. and Hawrot, E. (1985) Proc. Natl. Acad. Sci. USA 82, 8790–8794.
[16] Neumann, D., Barchan, D., Safran, A., Gershoni, J. and Fuchs, S. (1986) Proc. Natl. Acad. Sci. USA 83, 3008–3011.
[17] Dennis, M., Giraudat, J., Kotzyba-Hilbert, F., Goeldner, M., Hirth, C., Chang, J.Y. and Changeux, J.P. (1986) FEBS Lett. 207, 243–249.
[18] Carey, P.R. (1982) Biological Applications of Raman and Resonance Raman Spectroscopies, Academic Press, New York.
[19] Aslanian, D., Heidmann, T., Négrerie, M. and Changeux, J.P. (1983) FEBS Lett. 164, 393–400.
[20] Sobel, A., Weber, M. and Changeux, J.P. (1977) Eur. J. Biochem. 80, 215–224.
[21] Saitoh, T., Oswald, R.E. and Changeux, J.P. (1980) FEBS Lett. 116, 690–694.
[22] Heidmann, T., Oswald, R.E. and Changeux, J.P. (1983) Biochemistry 22, 3112–3127.
[23] Izzo, G.E., Jordan, F. and Mendelsohn, R. (1982) J. Am. Chem. Soc. 104, 3178–3182.
[24] Siamwiza, M.N., Lord, R.C., Chen, M.C., Takamatsu, T., Harada, I., Matsura, H. and Shimanouchi, T. (1975) Biochemistry 14, 4870–4876.
[25] Low, B.W., Preston, H.S., Sato, A., Rosen, L.S., Searl, J.E., Rudko, A.D. and Richardson, J.S. (1976) Proc. Natl. Acad. Sci. USA 73, 2991–2994.
[26] Scheule, R.K., Van Wart, H.E., Valle, B.L. and Scheraga, H.A. (1980) Biochemistry 19, 759–766.
[27] Kitagawa, T., Azuma, T. and Hamaguchi, K. (1979) Biopolymers 18, 451–465.
[28] Harada, I., Miura, T. and Takeuchi, H. (1986) Spectrochim. Acta 42A, 307–312.
[29] Thomas, G.J., Prescott, B. and Day, L. (1983) J. Mol. Biol. 165, 321–356.
[30] Oswald, R., Heidmann, T. and Changeux, J.P. (1983) Biochemistry 22, 3128–3136.
[31] Aslanian, D., Gróf, P., Bon, S., Masson, P., Négrerie, M., Chatel, J.-M., Balkanski, M., Taylor, P. and Massoulié, J. (1991) (submitted).
[32] Stroud, R.M. and Finer-Moore, J. (1985) Annu. Rev. Cell. Biol. 1, 317–351.
[33] Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Furutani, Y., Hirose, T., Asai, M., Inayama, S., Miyata, T. and Numa, S. (1982) Nature 299, 793–797.
[34] Devillers-Thiéry, A., Giraudat, J., Bentaboulet, M. and Changeux, J.P. (1983) Proc. Natl. Acad. Sci. USA 80, 2067–2071.
[35] Van Wart, H.E. and Scheraga, H.A. (1976) J. Phys. Chem. 80, 1812–1832.
[36] Van Wart, H.E., Cardinaux, F. and Scheraga, H.A. (1976) J. Phys. Chem. 80, 625–630.
[37] Proniewicz, L.M., Bajdor, K., Bruha, A., Kincaid, J.R. and Nakamoto, K. (1987) in: Laser Scattering Spectroscopy of Biological Object. Studies in Physics and Theoretical Chemistry, vol. 45 Elsevier, Amsterdam, pp. 211–218.
[38] Lord, R.C. and Yu, N.T. (1970) J. Mol. Biol. 51, 203–213.
[39] Rotschild, K.J., Andrew, J.R., De Grip, W.J. and Stanley, H.E. (1976) Science 191, 1176.
[40] Kao, P. and Karlin, A. (1986) J. Biol. Chem. 261, 8085–8088.