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Selection of Ion Channel Elements in the Serine and Aspartate Methyl-accepting Chemotaxis Proteins of Bacteria

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Two plausible, transmembrane ion channel elements (These "elements" are α-helical sequences of 24 amino acids in which polar, hydrophilic side chains occupy one side and hydrophobic side chains the other) have been identified in the serine chemoreceptor-methyl-accepting chemotaxis protein (MCP)(SerR) of E.coli and the aspartate chemoreceptor-MCP (AspR) of S.typhimurium. That the chemoreceptor might serve as, or activate, an ion channel is supported strongly by the occurrence of membrane depolarization, specific peptide methylation and neurotoxin inhibition of response in the chemotaxis of S.aurantia (E.P.Greenberg, refs.13-18].

The events which underlie the phenomenon of chemotaxis in bacteria have been elucidated over the past 15 years. [1-7] The sensory transducers for many stimuli are methyl-accepting chemotaxis proteins (MCPs), of which there appear to be 4 in E.coli and S.typhimurium, corresponding to the gene products of tsr, tar, trg, and tap. In at least two cases, the serine chemoreceptor (SerR) of E.coli [8] and the aspartate chemoreceptor (AspR) of S.typhimurium [9], the MCPs are also the chemoreceptors, and are transmembrane proteins. The complete amino acid sequences for both SerR and AspR have now been reported, but neither the functional portions of the receptor protein nor nature of the signal could be deduced from the sequence.

The speed with which the signal is transferred from the receptor to the motor (ca.200 msec [10]) makes a relatively small molecule, possibly a cation, a candidate for the signal. A potential change would be expected if a suitable cation entered (or left) the cell. However, attempts to demonstrate potential changes associated with chemotaxis in E.coli had been equivocal or negative until recently. [11,12] Previously, Greenberg [13-18] has shown a strong parallelism between depolarization of the membrane and chemotactic behavior in

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Spirochaete aurantia. A transient change in fluorescence, corresponding to membrane depolarization, could be correlated with a chemotactic response. In addition, an ionophore like valinomycin, which equalizes certain cation concentrations, especially K+, across the membrane but not nigericin, which affects the pH gradient across the membrane causes the loss of chemotaxis. [15] Tetrodotoxin, which blocks Na+ channels, and aconitine and sea anemone toxin, which prolong the open time of sodium channels, strongly inhibit chemotaxis to D-xylose. [18] Inhibition of chemotaxis by the ionophore A23187 suggests that a calcium channel might be involved at some step in the process. [18] The mechanism of aconitine action has been explained in terms of single group rotation theory elsewhere. [19] It thus became important to investigate the possibility that the serine and aspartate chemoreceptors were cation channels.

We have shown that ion "channel elements" (These "elements" are α-helical sequences of 24 amino acids in which polar, hydrophilic side chains occupy one side and hydrophobic side chains the other) may be selected from the amino acid sequences for the subunits of the acetylcholine receptor (AChR) and that an ion channel may be constructed from such elements. [20-23] One looks for mixed hydrophobic-hydrophilic sequences in which helix positions 1,4,8,11..are occupied by suitable groups, either charged, or polar. The fairly narrow definitions of AChR channel element units have been extended for the bacterial chemoreceptors to include polar side chains: amide groups (asn and gln)[Cf. the alamethicin channel [24]], the his imidazolium ion [SerR unit] and a thr hydroxy group [AspR unit].

The bacterial chemoreceptor channel elements were thus not so readily selected as those of the α-subunit of the AChR. Nevertheless, we have been able to tentatively identify two plausible channel elements in each chemoreceptor. The channel elements are illustrated in Fig. 1 for AspR and SerR. The amino acids which constitute the set of charged or polar groups which might line up along the α-helix are marked with *.

Aspartate Binding Protein

30		158gln		254		212rg+	
29	*	159asn	*	255gl-		211	
28	¦	160a	¦	256his		210g	
27g	¦	161	¦	257		209	
26g	¦	162g	¦	258gln		208	
25s	*	163gl-	*	259rg+		207	
24	¦	164a	¦	260s		206	
23	¦	165	¦	261		205	
22gln	¦	166g	¦	262		204	
21	*	167asn	*	263as-		203	
20	¦	168	¦	264t		202	
19a	¦	169a	¦	265		201	
18	*	170rg+	*	266t		200	
17	¦	171	¦	267gln		199	
16g	¦	172s	¦	268		198a	
15	*	173gl-	*	269rg+		197	
14	¦	174asn	¦	270gl-		196	
13	¦	175	¦	271g		195g	
12	¦	176	¦	272s		194	
11	*	177rg+	*	273as-		193gln	
10	¦	178gln	¦	274a		192	
9t	¦	179t	¦	275		191gln	
8	¦	180	¦	276		190a	
7	*	181as-	*	277s		189	
						188rg+	
H1	**H2**			**H4**		**H3**	

Serine binding protein

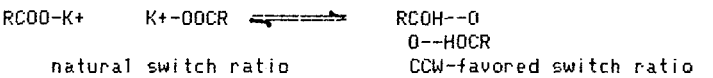
30		157rg+	*	244as-		214	
29	*	158as-	¦	245g		213g	
28	¦	159	¦	246s		212	
27g	¦	160rg+	¦	247asn		211	
26g	*	161asn	*	248gl-		210	
25s	¦	162g	¦	249		209a	
24t	¦	163	¦	250g		208	
23	¦	164gl-	*	251gln		207	
22gln	*	165+	¦	252		206	
21		166gln	¦	253a		205a	
20		167	*	254gl-		204	
19g		168	¦	255s		203	
18		169a	¦	256		202	
17		170	¦	257rg+		201	
16a		171	*	258his		200	
15	*	172gl-	¦	259		199	
14	¦	173gln	¦	260gln		198g	
13	¦	174asn	¦	261g		197	
12	¦	175as-	*	262gl-		196	
11	*	176rg+	¦	263		195	
10s	¦	177	¦	264		194	
9t	¦	178his	*	265rg+		193	
8	*	179as-		266t		192a	
7		180		267		191gln	
H1		**H2**		**H4**		**H3**	

Fig.1 The transmembrane ion channel elements and hydrophobic helices for the Salmonella typhimurium aspartate receptor (AspR). [left] (Russo,A.F. and Koshland,D.E., Science 220, 1016-1020 (1983)). The methylation sites (glu-, directly or derived from gln) are 294, 295, 296, 301, 302, 483, 484, 490, 491, 499, 500, 501. The transmembrane ion channel elements and hydrophobic helices for the Escherichia coli serine receptor (SerR). [right] (Boyd,A., Kendall,K. and Simon,M.I., Nature 301, 623-626 (1983)) The methylation sites (glu-, directly or derived from gln) are 296, 297, 298, 303, 304, 310, 311, 492, 493, 502, 503. The helices for each receptor are marked in order of occurrence, H1-H4. The nonstandard abbreviations are:+, lys; g, gly; s,ser; a, ala; gl-, glu; rg+, arg; t, thr; as-, asp. Unmarked amino acids are hydrophobic [trp, tyr, leu, ile, val, met]. The * indicates a hydrophilic unit within a channel element.

The ion channel is constructed of the channel elements. If there were only one channel element per molecule, the C-terminus would be outside the membrane. This is considered unlikely in view of the location of the sites for methylation, which are intracellular. Thus, there must be two channel elements. The ion channel is possibly trimeric on geometric grounds, and may be, as implied by the MCP-crosslinking of Dahlquist, even tetrameric. [25] The AChR is thought to have seven channel elements, so that 6 - 8 channel elements seems reasonable, but a dimer(4 channel elements) is also possible.

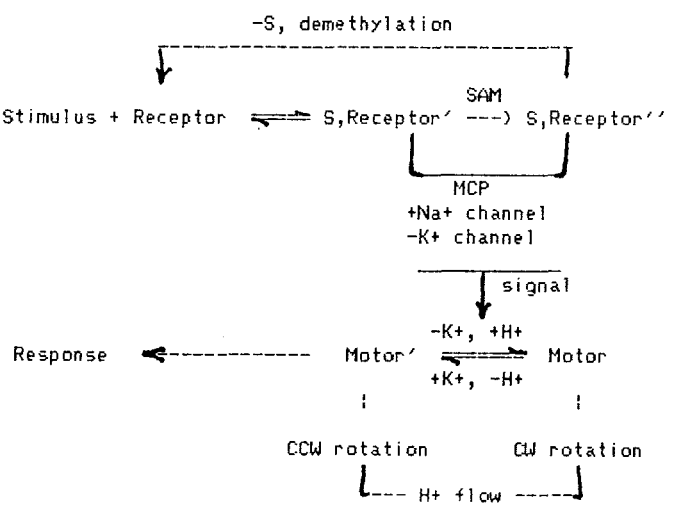
The simplest hypothesis is to assume that the channel carries Na+, and that the commencement of depolarization activates K+-channels. The signal

transmitted to the motor would then be a lowered K+ activity, leading to a dissociation of a K+-complex at the motor switch. [Greenberg, personal communication] Protons could then occupy the switch site, which we shall treat as carboxylate groups, and overcome repulsion between two such groups. This partial molecular model for the motor switch is compatible with asynchrony in switching between different flagella [26] and might be useful in explaining the directions for motor motion favored for inward proton motion (CCW) or outward proton motion (CW). [27] (Scheme I)



SCHEME I

Channel operation would be modulated by methylation of carboxyl groups in the cytoplasmic portion of the MCPs, possibly through releasing blocking groups held out of the channel by hydrogen bonding. Demethylation, as observed for B.subtilis, could also affect channel operation. [28, 29] Methylation is apparently also involved in the chemotaxis of the non-enteric bacteria, Pseudomonas aeruginosa [30] and Spirochaeta aurantia [18]. A modified scheme for chemotaxis which includes the K+ ion effect is shown as Scheme II. The scheme presumes parallels in the mechanism for all the organisms from which the individual pieces of information have been derived.



SCHEME II

SAM = S-adenosylmethionine; CCW = counterclockwise; CW = clockwise; MCP = methyl-accepting chemotaxis protein

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References

[1] Adler,J. (1975). Ann.Rev.Biochem. 44, 341-356.
[2] Clarke,S. and Koshland,D.E.,jr. (1979)J. Biol. Chem. 254, 9695-9702
[3] Berg,H.C. (1975) Ann. Rev. Biophys. Bioengineer.4, 119-136
[4] Khan,S. and Macnab,R.M. (1980)J. Mol. Biol. 138, 563-597
[5] Silverman,M. and Simon,M.(1977) Proc.Nat'l.Acad.Sci.USA 74, 3317-3321
[6] Parkinson,J.S. (1978) Cell 15, 1221-1230
[7] Chelsky,D. and Dahlquist,F.W. (1981) Biochemistry 20, 977-982
[8] Boyd,A., Kendall,K. and Simon,M.I. (1983) Nature 301, 623-626
[9] Russo,A.F. and Koshland,D.E. (1983) Science 220, 1016-1020
[10] Segall,J.E., Manson,M.D. and Berg,H.C. (1982) Nature 296, 855-857
[11] Eisenbach,M. (1982) Biochemistry 21, 6818-6825
[12] Eisenbach,M., Raz,T. and Ciobotariu,A.(1983) Biochemistry 22, 3293-3298
[13] Greenberg,E.P. (1977)J.Bacteriol. 130, 485-494
[14] Goulbourne,jr.,E.A. and Greenberg,E.P.(1980)J.Bacteriol.143, 1450-1457
[15] Goulbourne,jr.,E.A. and Greenberg,E.P.(1981)J.Bacteriol. 148, 837-844
[16] Goulbourne,jr.,E.A. and Greenberg,E.P.(1983)J. Bacteriol. 153, 916-920
[17] Kathariou,S. and Greenberg,E.P. (1983)J.Bacteriol.,in press
[18] Goulbourne,jr.,E.A. and Greenberg,E.P.(1983) J.Bacteriol., in press
[19] Kosower,E.M., cited in Witkop,B. (1983) in The Alkaloids, Vol.21, ed., Broosi,A., Academic Press, New York, pp.224-226
[20] Kosower,E.M. (1983) Biochem. Biophys. Res. Comm. 111, 1022-6
[21] Kosower,E.M. (1983) FEBS Letters, 155, 245-247
[22] Kosower,E.M. (1983) FEBS Letters, in press.
[23] Kosower,E.M. (1984) Biophysical J., in press
[24] Fox,R.O.,jr. and Richards,F.M. (1982) Nature 300, 325-330
[25] Chelsky,D. and Dahlquist,F.W. (1980) Biochemistry 19, 4633-4639
[26] Macnab,R.M. and Han,D.P. (1983) Cell 32, 109-117
[27] Khan,S. and Berg,H.C. (1983) Cell 32, 913-919
[28] Goldman,D.J., Worobec,S.W., Siegel,R.B., Hecker,R.V. and Ordal,G.W. (1982) Biochemistry 21, 915-920
[29] Ordal,G.W., Nettleton,D.G. and Hoch,J.A. (1983) J. Bacteriol. 154, 1088-1097
[30] Craven,R.C. and Montie,T.C. (1983) J. Bacteriol. 154, 780-786