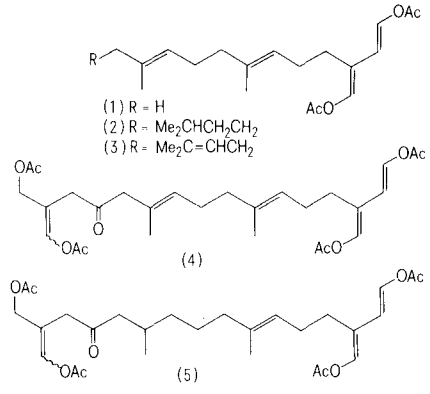
peared in the 70 e.v. EI-MS of **3** which showed the 1st observable ion at m/e 328 ($M^+ - 60$) with other prominent ions at m/e 286, 191, 149, 109, 69, 43. Comparison of the ^1H and ^{13}C-NMR data of **3** with that of **1** and **2** established the structure of **3**. The ^1H-NMR-spectrum of **3** in CCl_4 showed resonances at δ 7.32 (1H, d, J = 12 Hz). 7.08 (1H, bs), 5.82 (1H, d, J = 12 Hz), 5.05 (3H, m), 2.08 (3H, s), 2.06 (3H, s), 1.84 (3H, bs) and 1.59 (9H, bs) and the ^{13}C-NMR-spectrum in $CDCl_3$ gave resonances at δ 167.5 (s), 167.1 (s), 135.9 (s), 135.4 (s), 134.2 (d), 131.0 (s), 124.3 (d), 124.1 (d), 123.2 (d), 121.1 (d), 113.2 (d), 39.6 (t), 26.7 (t), 26.6 (2C, t), 25.6 (q), 25.3 (t), 20.5 (2C, q), 17.6 (q), 16.0 (2C, q). UV- and IR-spectra of **3** (ν_{max} 1760 cm^{-1}; λ_{max} 252 nm (log ε 4.49) were almost identical to those of **1** and **2**.

Chlorodesmin (**4**) gave a very weak molecular ion in the CI-MS and the molecular formula was extrapolated as $C_{28}H_{38}O_9$ from high resolution data on the 1st major fragment ion at m/e 458 ($M^+ - 60$). The ^1H-NMR-spectrum demonstrated the presence of a diacetoxybutadiene moiety with signals at δ (CCl_4) 7.34 (1H, d, J = 12 Hz), 7.09 (1H, bs) and 5.85 (1H, d, J = 12 Hz) and the appearance of dominant ions at m/e 191 ($C_{12}H_{15}O_2$) and 149 ($C_{10}H_{13}O$) parallelled the MS behaviour of **1**–**3**. The remainder of the ^1H-NMR-spectrum of **4** consisted of resonances at δ 7.01 (1H, bs), 5.16 (2H, m), 4.64 (2H, s), 3.09 (2H, s), 3.00 (2H, s), 2.13 (3H, s), 2.10 (3H, s), 2.08 (3H, s), 1.95 (3H, s) and 1.60 (6H, bs). The ^{13}C-NMR-spectrum of **4** [δ ($CDCl_3$) 205.5 (s), 169.8 (s), 167.1 (s), 166.7 (2C, s), 135.5 (d), 135.2 (s), 135.2 (d), 133.8 (d), 129.2 (d), 128.2 (s), 123.1 (d), 120.5 (s), 113.5 (s), 112.6 (d), 59.2 (t), 52.7 (t), 41.4 (t), 38.6 (t), 26.0 (t), 26.0 (t), 24.7 (t), 20.0 (4C, each q), 15.7 (q) and 15.3 (q)] demanded the presence of 1 ketonic carbonyl, 4 ester carbonyls, 4 trisubstituted and 1 disubstituted double bonds, and a $-CH_2-O$ grouping. Thus **4** must be acyclic. Fully coupled ^{13}C-NMR-spectra showed that the coupling constants of the doublets at δ 135.2, 135.0 and 133.8 were between 189 and 193 Hz attributable to the presence of 3 enol acetate groups in **4** and the absence of a methyl signal at about δ 25 showed the absence of a Z terminal methyl. The majority of the spectrum was very similar to that of (**3**) suggesting that the 3rd enol acetate, the $-CH_2OAc$ group and the nonconjugated ketone were grouped at the opposite end of the diterpene chain to that of the diacetoxydiene moiety.



2 proton singlets at δ 4.64, 3.09 and 3.00 in the ^1H-NMR-spectrum of (**4**) indicated the presence of 1 allylic acetoxymethyl group and 2 doubly allylic methylenes in the molecule and only structure **4** satisfies all spectral data.

Dihydrochlorodesmin (**5**) had mass spectral and NMR features very similar to those of (**4**). Thus dominant mass spectral ions appeared at m/e 191, 149 and 91 as in (**4**) and the ^1H-NMR-spectrum [δ ($CDCl_3$) 7.37 (1H, d, J = 12 Hz), 7.10 (1H, bs), 7.06 (1H, bs), 5.88 (1H, d, J = 12 Hz) 5.14 (1H, bt, J = 7 Hz), 4.72 (2H, s), 3.08 (2H, s), 2.12 (6H, s), 2.10 (3H, s), 1.99 (3H, s), 1.53 (3H, bs), 0.88 (3H, d, J = 7 Hz)] was similar to that of (**4**) with the exception that 1 doubly allylic methylene group was missing and a $-C(Me)=CH-$ grouping now appeared as a $-CH(Me)-CH_2-$group. The ^{13}C-NMR-spectrum [δ ($CDCl_3$) 207.0 (s), 170.4 (s), 167.6 (s), 167.2 (s), 167.0 (s), 135.5 (2C, each d), 134.2 (d), 123.4 (d), 121.0 (s), 114.0 (s), 113.2 (s), 113.2 (d), 59.7 (t), 49.5 (t), 43.7 (t), 39.5 (t), 36.4 (t), 29.0, 26.5, 25.2, 20.5 (4C, q), 19.7 (q), 15.7 (q)] also was fully in accord with structure (**5**) for dihydrochlorodesmin.

Attempted hydride reduction, hydrogenation or gentle acid and base hydrolysis of both **4** and **5** resulted in complex product mixtures and paucity of material precluded further chemical work.

1 A.J. Blackman and R.J. Wells, Tetrahedron Lett. *1978*, 3063.
2 V. Amico, G. Oriente, M. Piatelli, C. Tringali, E. Fattorusso, S. Magno and L. Mayol, Tetrahedron Lett. *1978*, 3593.
3 H.H. Sun and W. Fenical, Tetrahedron Lett. *1979*, 689.
4 RRIMP Museum Specimen FN 1912/000.

Concerning the anchoring of acetylcholinesterase in biomembranes

H. Grossmann, K.-P. Ruess and M. Liefländer

Chemisches Institut der Universität Regensburg, Universitätsstrasse 31, D-8400 Regensburg (Federal Republic of Germany)
22 March 1979

Summary. Highly purified acetylcholinesterases from various sources do not contain hydroxyproline and hydroxylysine, indicating the absence of a collagenlike 'tail'. The enzymes examined are therefore bound to the membrane in a different way compared with acetylcholinesterase isolated from electric organ.

At the present time a great deal of interest is focussed on the possibility that highly purified acetylcholinesterase may contain hydroxyproline and hydroxylysine. A percentage of 0.3–0.8% of these amino acids is considered to show that the acetylcholinesterase in question contains a collagen-like 'tail'. Highly purified 18S- and 14S-molecular forms of acetylcholinesterase from electric organ tissue do contain such significant amounts of hydroxyproline and hydroxylysine, although a more detailed description of the collagen-like 'tail' cannot yet be given. The putative role of the 'tail' in anchoring acetylcholinesterase to the fibrillar matrix of the basement membrane (even in the case of mammalian muscle endplate) and in self-association of the various molecular forms at low ionic strength is open to speculation and is under investigation[1].

The electric organ is a highly specialized tissue, which evolved from muscle tissue with its known collagen content. We were interested in analyzing our several highly

Hydroxyproline content of various highly purified acetylcholinesterases

AcChE from	Specific activity (U/mg)	Mode of solubilization	Hydroxyproline
Bungarus multicinctus	4290	water soluble	0.023 ± 0.02%
Naja naja atra	5470	water soluble	0.024 ± 0.02%
Human erythrocytes	4270	0.2% Triton X-100	0.037 ± 0.03%
Bovine erythrocytes	4130	0.5% Triton X-100	0.043 ± 0.03%
Bovine nucleus caudatus	4250	0.6–0.8% Triton X-100	0.035 ± 0.03%
Electrophorus electricus[1,7] (14S + 18S-form)	-	1 M NaCl	0.83 ± 0.13%
Electrophorus electricus[1,7] (11S-form)	-	proteolytic digestion	0.1 ± 0.06%

purified acetylcholinesterases from various other sources for hydroxyproline and hydroxylysine.

Material and methods. The acetylcholinesterases shown in the table (except those from electric fish) were solubilized by the method mentioned and purified to the indicated high specific activities by repeated affinity chromatography[2, 3]. Purity of the enzymes was demonstrated by electrophoresis under non-denaturing conditions, and by the fact that specific activity did not increase on further purification steps like affinity chromatography, electrophoretic[4] – or centrifugal[5] – separation of single enzyme forms. Hydroxyproline was analyzed by a modified method from Bondjers and Björkerud[6], hydroxylysine using an aminoacid analyser (Biocal BC 201).

Results and discussion. Hydroxylysine was absent in all acytylcholinesterases examined. The table shows that none of the enzymes contains hydroxyproline in such a significant amount similar to the 14S/18S-forms of electric fish acetylcholinesterase. We therefore suppose that the acetylcholinesterases examined do not possess a collagen-like 'tail', although they are extracted and purified under non-proteolytic conditions like the 14S/18S-forms of electric fish acetylcholinesterase. With exception of the 2 water-soluble 'snake' enzymes, the acetylcholinesterases are membraneous proteins. The way of anchoring acetylcholinesterase to the membrane, however, must be quite different from what is found in electric tissues. This assumption is supported by the fact that very different methods must be applied for solubilizing the acetylcholinesterases mentioned. Whereas the collagen-like 'tail' containing acetylcholinesterase from *Electrophorus electricus* can easily be solubilized with 1 M NaCl, high salt concentration severely inhibits solubilization of acetylcholinesterase from bovine nucleus caudatus. This enzyme slowly solubilizes to a large amount at very low salt concentration, whereas under the same conditions, solubilization of acetylcholinesterase from human erythrocytes does not take place and acetylcholinesterase from bovine erythrocytes is only dispersed in particles. A unifying concept of anchoring acetylcholinesterase to the membrane seems to be impossible.

1 L. Anglister and I. Silman, J. molec. Biol. *125*, 293 (1978).
2 K.-P. Ruess, M. Weinert and M. Liefländer, Z. physiol. Chem. *357*, 783 (1976).
3 H. Grossmann and M. Liefländer, Z. physiol. Chem. *356*, 663 (1975).
4 K.-P. Ruess and M. Liefländer, Hoppe-Seylers Z. physiol. Chem. *358*, 1543 (1977).
5 P. Kramer, K.-P. Ruess and M. Liefländer, unpublished results.
6 G. Bondjers and S. Björkerud, Analyt. Biochem. *52*, 496 (1973).
7 L. Anglister, S. Rogozinski and J. Silman, FEBS Letters *69*, 129 (1976).

The halophilic properties of pyruvate kinase from *Vibrio costicola*, a moderate halophile

Eveline de Médicis and B. Rossignol

Department of Biochemistry, Faculty of Medicine, Sherbrooke (Quebec JIH 5N4, Canada), 19 March 1979

Summary. Pyruvate kinase from *Vibrio costicola*, a moderate halophile, appears to be adapted to functioning in the presence of salt. Its stability depends on the ionic strength of the medium. The amino acid composition resembles that of other halophilic enzymes. It is proposed that the halophilic pyruvate kinase utilizes preferentially the Mn^{++} cofactor which forms more stable complexes in the presence of physiological concentrations of salt.

Vibrio costicola is a moderately halophilic bacterium having an intracellular salt concentration of 0.95 M Na^+ and 0.66 M K^+ [1]. The cytoplasmic pyruvate kinase purified from the bacterium appears to be inhibited by NaCl and KCl to an extent which is not compatible with the physiological conditions[2]. The stability of pyruvate kinase, however, is so dependent upon the presence of salt in the solvent that it is improbable that the enzyme is sequestered in a compartment at low ionic strength in vivo. The enzyme is stable in 30% (v/v)glycerol at room temperature but in solution devoid of glycerol, the enzyme is inactivated within a few days. The enzyme is less stable at low ionic strength and requires salt for increased stability. This characteristic is shared by other halophilic enzymes purified from extremely halophilic bacteria. It has been proposed that the amino-acid composition of halophilic proteins could be different from that of non-halophilic proteins, in order to explain the adaptation of the 3-dimensional structure to salt environments. The amino acid composition of pyruvate kinase from *Vibrio costicola* has been determined. Purified pyruvate kinase[2] was hydrolyzed in sealed tubes with 5.6 N HCl at 110 °C for periods of 24, 48 and 72 h. The hydrolysates were analyzed on a Technicon TSM using the type A resin. Amino-acids values were corrected with an internal standard of norleucine. The values of Asx, Arg and Ser were extrapolated to zero time and the value of Thr was that measured at 72 h of hydrolysis. A peak corresponding to ornithine was found and its content was checked by mass spectrometry. Table 1 shows the amino-acid composition of pyruvate kinase from *Vibrio costicola* and the amino-acid compositions of pyruvate kinases from other sources for comparison. Ornithine is a