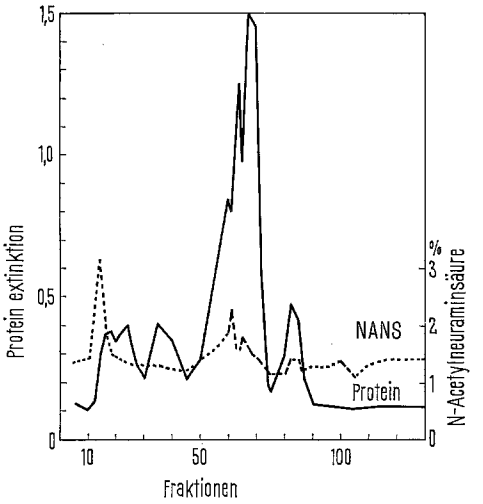


Elutionsverlauf des durch Pronase freigesetzten Enzyms an Sephadex G-50. Fraktionierung des Überstandes vom Gesamthomogenat nach Zentrifugation bei 65000 × *g*/25 min. Eluiert mit 0,05 *M* Tris-HCl-Puffer pH 7,0; 5 ml-Fraktionen. Durchgezogene Linie zeigt den Verlauf der Proteinelution, durchbrochene Linie gibt die Neuraminidase-Aktivität an. Ordinate links: Extinktion des Proteins, bestimmt nach Lowry[10]. Ordinate rechts: freigesetzte Menge N-Acetylneuraminsäure (NANS), bestimmt nach Aminoff[11] und bezogen auf den Neuraminsäuregehalt des Substrates. Als Substrat diente ein gereinigtes und dialysiertes Gangliosidgemisch mit 16% N-Acetylneuraminsäure.

Ammonsulfatfraktion wurde bis zur Dreiviertelsättigung mit kristallisiertem Ammonsulfat versetzt. Das Sediment wurde in 5 ml 0.2 *M* K-Acetatpuffer (pH 4.3) gelöst und ebenfalls 16 h lang bei 4 °C gegen 0.2 *M* K-Acetatpuffer (pH 4.3) dialysiert. Die dialysierten Proteinlösungen wurden in insgesamt 6 ml 0.2 *M* K-Acetatpuffer (pH 4.3) suspendiert. Jeweils 1 ml dieser Lösungen wurde zu 1 mg Gangliosid hinzugegeben. Nach zweistündiger Inkubation bei 37 °C bestimmten wir die freigesetzte Menge N-Acetylneuraminsäure in 0.2 ml-Proben des Inkubationsansatzes nach der Methode von Aminoff[11]. Unter denselben Bedingungen wurden wiederum zwei Kontrollbestimmungen durchgeführt[12].

Summary. The release of membrane-bound brain neuraminidase from brain homogenate by proteolytic digestion with trypsin, α-chymotrypsin and pronase is described. Trypsin shows the highest solubilizing effect. On the basis of the ammoniumsulphate precipitation the pronase-released neuraminidase has the lowest molecular size.

W. Gielen

Pharmakologisches Institut
der Universität Köln, Postfach 1709,
D-5 Köln-Lindenthal (Deutschland), 17. Dezember 1969.

[12] Mit Unterstützung durch das Landesamt für Forschung des Landes Nordrhein-Westfalen.

Action of Sodium Dodecyl Sulfate on Electron Transport Enzymes of *Rhodospirillum rubrum*

In an attempt to elaborate relations between molecular architecture and functioning of bacterial membranal systems, the effects of detergents on the particulate electron transport system of *R. rubrum* have been studied. The enzymes of this system were shown to exhibit a different activation-inactivation behaviour in the presence of the detergents tested, sodium deoxycholate (DOC)[1] and Triton X-100[2]. A particulate NADH-dehydrogenase was partly solubilized by DOC and has subsequently been characterized[3]. The present study is concerned with the action of the strong anionic detergent sodium dodecyl sulfate (SDS) on the *R. rubrum* system.

Methods. The methods used for cultivation of cells, for the preparation of the electron transport particulate fraction and the conditions for assaying the different electron transport enzymes were those described in detail in previous papers[1,3–5]. Frozen cell material was used for the experiments to be described[2].

Results and discussion. The ability of SDS to solubilize the membraneous system of *R. rubrum* was found to be quite similar (with respect to detergent/membrane protein ratio and extent of solubilization) to that of DOC and Triton X-100[2]. Thus no difference between anionic detergents (DOC and SDS) and a non-ionic detergent (Triton X-100) is seen in this respect.

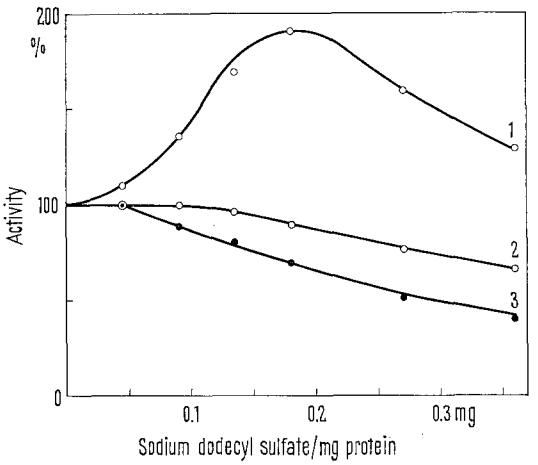
In the Figure the activities of several electron transport components are depicted in their response to increasing concentrations of SDS at 0 °C. The NADH oxidase system, as it was already seen with the other detergents, is the most sensitive activity. Succinate-cytochrome c-reductase also becomes inactivated, while NADH-cytochrome c-reductase is stimulated by approximately 100% before higher concentrations of detergent inactivate the enzyme. Here profound differences exist between the 3 detergents tested. With DOC NADH- and succinate-cytochrome c-reductase both become stimulated[1], whereas in the presence of Triton X-100 the activity of succinate-cytochrome c-reductase is increased while NADH-cytochrome c-reductase is rapidly inactivated[2]. Under the conditions of the experiments none of the activities is rendered into a soluble form. It should be noted that the NADH oxidase of mycoplasma was recently shown to be extremely stable as compared to the *R. rubrum* enzyme. A SDS concentration 3 times that which, at 0 °C, causes 50% inactivation of the latter enzyme will at 37 °C still activate the mycoplasma enzyme by over 100%[6].

The solubilizing effect of the detergents is probably a result of a displacement of lipid from its intimate association with the membrane protein so that structural and functional integrity can no longer be maintained. The complex behaviour of the activities just mentioned indicates that by such dispersal different effects on the membranal system and its components can be produced by the individual detergents. Those effects which manifest in altered enzymatic activities might modify the substrate or acceptor binding sites of the membrane enzyme (e.g. increased accessibility causes stimulation

[1] M. Boll, Arch. Mikrobiol. *68*, 191 (1969).
[2] M. Boll, Arch. Mikrobiol., *71*, 1 (1970).
[3] M. Boll, Arch. Mikrobiol. *69*, 301 (1969).
[4] M. Boll, Arch. Mikrobiol. *62*, 94 (1968).
[5] M. Boll, Arch. Mikrobiol. *64*, 85 (1968).
[6] S. Razin, Z. Ne'eman and I. Ohad, Biochim. biophys. Acta *193*, 277 (1969).

of the activity as with Triton X-100 and succinate-cytochrome c-reductase or as with SDS and NADH-cytochrome c-reductase) or they affect the correct alignment of the enzyme within the complete electron transport system.

In the presence of DOC or Triton X-100, further inactivation beyond the degree obtained by the detergent concentration employed proceeds very slowly and the stimulated activities are practically stable[1,2]. In contrast, samples containing SDS become increasingly inactivated. With a detergent/membrane protein ratio of 0.18 the activities of the Figure (which remain unchanged for 15 min) become inactivated within 3 h: NADH-cytochrome c-reductase by 25%, succinate-cytochrome c-reductase by 75% and NADH oxidase by 80%. Attempts to minimize this inactivation by dialyzing the samples (3 and 6 h against frequent changes of 0.05 *M* phosphate buffer pH 7.5 in the cold) had no effect, indicating an apparent firm bonding of the detergent with the membranes.



The effect of SDS on (1) NADH- and (2) succinate-cytochrome c-reductase and on (3) NADH oxidase of the electron transport particulate fraction of *R. rubrum*. SDS was added in the cold to electron transport particles (4.2 mg protein/ml) to obtain the desired detergent/membrane protein ratio. After 15 min at 0 °C activity was determined.

Inactivation of electron transport enzymes by SDS

mg SDS/ mg protein (min at 20 °C)	Inactivation (%)			
	NADH-cyto-reductase	Succinate-cyto-reductase	NADH dehydro-genase (DCPIP)	Succinate dehydro-genase
0.18 (10)	50	90	12	< 5
0.36 (10)	90	> 95	35	< 5
0.72 (10)	–	–	60	12
0.72 (30)	–	–	85	32

SDS was added to electron transport particles (4.2 mg protein/ml) at 20 °C and activities were determined after the stated time intervals. The values of the 30 min incubation were corrected for a slight inactivation without SDS.

The different stimulation-inactivation behaviour of NADH- and succinate-cytochrome c-reductase in the presence of Triton X-100 and SDS, besides indicating different modes of action of the 2 detergents, favours the idea that these effects are exerted on that part of the electron transport system where 2 distinct pathways exist: NADH → Coenzyme Q and succinate → Coenzyme Q. However neither is NADH dehydrogenase (DCPIP) stimulated nor does succinate dehydrogenase (phenazine methosulfate-DCPIP assay) become inactivated by appropriate concentrations of SDS. Only much higher detergent/membrane protein ratios would affect these activities. Because of the increasing insolubility of SDS at 0 °C, the experiments were carried out at 20 °C where inactivation, as to be expected, is more intensive (see NADH- and succinate-cytochrome c-reductase in the Figure and in the Table). The values of the Table demonstrate that succinate dehydrogenase is even less sensitive to SDS than NADH dehydrogenase. Provided that the determinations of the 2 activities with the artificial electron acceptors have physiological significance, this seems to exclude a direct influence of the detergent on the two flavoproteins of this part of the electron transport system. Glycerol or sucrose (0.5 *M*) which have in some instances been found to stabilize enzymatic activities in the presence of detergents[7] failed to show any protection of NADH- and succinate dehydrogenase.

Zusammenfassung. SDS hat die gleiche solubilisierende Wirkung auf das Membransystem von *Rhodospirillum rubrum* wie DOC und Triton X-100. NADH- und Succinat-Cytochrom-c-Reduktase des partikulären Elektronentransportsystems zeigen in Gegenwart von SDS jedoch ein sowohl von DOC als auch von Triton X-100 verschiedenes Aktivierungs-Inaktivierungsverhalten. Eine direkte Wirkung von SDS auf die beiden Flavoproteine NADH- und Succinat-Dehydrogenase im getrennten Anfangsbereich der Elektronentransportkette ist unwahrscheinlich.

M. Boll[8]

Lehrstuhl für Mikrobiologie, Institut für Biologie II der Universität Freiburg, D-78 Freiburg i. Br. (Germany), 25 March 1970.

[7] Z. Selinger, M. Klein and A. Amsterdam, Biochim. biophys. Acta *183*, 19 (1969).

[8] Supported by the Deutsche Forschungsgemeinschaft and by the Stiftung Volkswagenwerk. The author wishes to thank Prof. Dr. G. Drews for his interest in the work.

Electrical Membrane Constants of Sartorius Muscle Fibers from the South American Frog, *Leptodactylus ocellatus*

Materials and methods. Sartorius muscles were dissected and mounted as previously described[1]. The temperature in the nerve-muscle chamber was kept at 25 °C. Bathing solutions were prepared by adding 2.5 m*M* of KCl or CsCl per l to standard K-free Ringer (112 m*M* NaCl; 1.89 m*M* $CaCl_2$, 2.5 m*M* Na_2HPO_4; 0.5 m*M* NaH_2PO_4; pH 7.2). The osmolarities of all solutions were checked cryoscopically. Each number given below for membrane potential represents an average over measurements on 40 individual muscle fibers.