The Nature of Electrode Processes of Guanine and its Derivatives

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At negative potentials in the region of background electrolyte decomposition, a product arises on the mercury electrode in solutions of guanine and its derivatives. The product is probably in its reduced form. An oxidation of this product is responsible for the origin of anodic identification on oscillograms of dE/dt against E or of anodic polarographic waves.

Polarographic methods have furnished valuable information on the structure of nucleic acids. For the correct interpretation of the results obtained with polymeric nucleic acids it is necessary to know the mechanism of the electrode reactions of their individual components. Next to cytosine, whose behaviour on the dropping mercury electrode has already been elucidated, it is guanine that also takes part in the polarographic activity of nucleic acids. For this reason we tackled the task of studying more profoundly its electrode reactions.

Native and denatured deoxyribonucleic acid (DNA) yields in certain media on the oscillographic curves dE/dt against E an anodic indentation for the origin of which the guanine moiety of the DNA molecule is responsible. This anodic indentation, suitable for analytical use, produces both guanine and its nucleosides and nucleotides. The depth of the anodic indentation depends on the temperature (with rising temperature, the depth decreases) and on the direct current component of the polarization voltage (in incomplete polarization towards the potential of the right marginal point, i.e. in diminishing of the negative component, the indentation disappears). These facts have led us to the assumption that the anodic indentation is conditioned through an artefact at the potentials in the vicinity of the right marginal point, i.e. in the region of the background electrolyte decomposition. At the potential of the anodic indentation, oxidation of the artefact takes place. This assumption has been corroborated by the results of the experiments with deoxyguanilic acid on the hanging mercury drop electrode. The drop was first polarized with direct voltage towards potentials corresponding to the beginning of hydrogen elimination from the background electrolyte, whereupon the anodic polarographic curve was recorded. Deoxyguanilic acid yielded a peak, whose potential corresponded approximately to the potential of the anodic indentation.

In favour of our assumption also speak the results of the present experiments in which we availed ourselves of a Kalousek switch.

Results and Discussion

The dropping mercury electrode was alternately polarized with a negative constant voltage \(E_{\text{coul}}\) derived from a auxiliary potentiometer and with a voltage gradually rising from positive values (from the beginning of the anodic dissolution of the mercury) to negative values and derived from a polarographic wheel. The voltage was switched over with the aid of a relay of 6 c/s frequency and the current was recorded only at the time when the electrode was polarized with the voltage derived from the polarograph. At a sufficiently negative auxiliary constant voltage, deoxyguanosine (Calbiochem) yielded in a medium of 0.5 M ammonium formate plus 0.05 M ammonium phosphate an anodic

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wave, whose height at a given $p_H$ value depended on the values of the constant potential (Fig. 1). Thus, at a sufficiently high negative constant potential introduced on the dropping mercury electrode a product forms, which is then oxidized upon switching over to more positive potentials. This manifestation itself by the formation of an anodic wave, whose halfwave potential depends on the $p_H$ value of the medium (Fig. 2). When the constant potential is raised, the height of the anodic wave first rises and drops again after reaching a certain value (Fig. 1). This may be due to the fact that at highly negative potentials, an irreversible change sets in and the yielded product is not any more subjected to oxidation. These findings are in agreement with the results obtained on the hanging mercury drop-electrode\(^8\). Since the potentials at which reduction takes place lie in the region of hydrogen elimination from the background electrolyte, we have an explanation why guanosine is regarded as polarographically non-reducible\(^8\). However, we observed that it lowers the overpotential for hydrogen ion reduction by about 100 mV.\(^9\)

The dependence of the depth of the anodic indentation \(\nu \) for deoxyguanosine on \(p_H\) is in the form of a dissociation curve; the dependence of the height of the anodic wave at the introduction of an optimum constant voltage (i.e. of such a voltage where the wave height in the given medium is maximal) has a similar shape (Fig. 2). The apparent (polarographic) dissociation constant \(p K_a' = 6.9\) estimated from the curve (Fig. 2), compared with the dissociation constant for guanosine \(p K_a = 1.6\)\(^9\), is shifted towards higher values, probably due to the recombination with $H^+$ ions. The active form in the electrode process taking place at negative constant potential is suggested to be a protonized form of guanosine.

Through macroscale electrolysis of guanosine at a controlled potential (about $-1.8 \text{ V}$) also arises a product yielding an anodic oxidation wave, whose half-wave potential corresponds to the half-wave potential of the anodic wave obtained by means of the Kalousek switch. On the curves $dE/dt$ against $E$ the product yields an anodic indentation even at incomplete polarization towards the potential of the right marginal point. Its position is identical with the position of the indentation produced by guanosine. The character of the product and the mechanism of its formation are the subject of further studies.
