Cathode-Ray Polarography of Insulin

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Brdicka has shown that proteins give rise to characteristic polarographic double waves in the presence of Co^{2+} ions (Brezina & Zuman, 1958). These waves have been attributed to a catalytic reaction occurring at the dropping-mercury electrode, although the theoretical aspects of the reaction are still not well understood (Muller, 1963). Attempts have been made to utilize this reaction as a diagnostic test for cancer (Stricks, Kolthoff, Bush & Juroda, 1953).

Conventional polarography has been used to

study insulin in different supporting electrolytes in the absence of Co^{2+} ions (Christian, Knoblock, Purdy & Mertz, 1963). The polarographic waves obtained were not given, only the half-wave potentials. We have repeated this work with samples of insulin from three different sources, but the resulting polarograms are complicated by maxima and irregular diffusion phenomena.

In cathode-ray polarography a fast potential sweep is applied during the lifetime of one single mercury drop. The resulting peaked waves, due to

Fig. 1. Cathode-ray polarograms of ten-times-recrystallized insulin obtained with a Southern Analytical A1670 Davis Polarograph. (a) and (d) $10\mu\text{M}$ -Insulin in 0.1M -HCl at $\frac{1}{75}$ times maximum sensitivity; (b) and (c) $10\mu\text{M}$ -insulin in 0.1M -KCl at $\frac{1}{30}$ and $\frac{1}{250}$ times maximum sensitivity respectively.

reduction or oxidation of the electroactive species at the surface of the mercury drop, are usually better resolved and very much more sensitive than the corresponding conventional polarograms.

The cathode-ray polarograms observed for reduction of ten-times-recrystallized insulin ($10\mu\text{M}$) are shown in Figs. 1(a)–1(c). Fig. 1(a) shows a reduction wave at a potential of -0.23 V (with respect to Ag/AgCl reference electrode), which is possibly due to the reduction of the disulphide group in insulin. The potential for this reduction wave is very dependent on the H^+ ion concentration, as has been observed for the reduction of the disulphide group in cystine (W. Lee, unpublished work). At more negative potentials further reduction waves are observed (Figs. 1b and 1c). By reversing the direction of the potential sweep it is possible to observe an anodic wave, which can be attributed to reoxidation of the reduced species formed at the mercury drop.

Fig. 1(d) shows the anodic wave corresponding to the reverse process of the cathodic wave in Fig. 1(a). The separation between these peak potentials in millivolts (Figs. 1a and 1d) corresponds to a reversible two-electron reaction, occurring at the dropping-mercury electrode.

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