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# Colour Change of Prodigiosin

OBSERVATIONS of the colour of *Serratia marcescens* colonies and of chromatographic behaviour of *Serratia* pigments recently led Allen[1] to suggest that "*p*H is not the only factor involved in the colour change of prodigiosin from red to orange".

The red colour of *Serratia* colonies even in moderately alkaline media is not surprising, because relatively large amounts of serratamic acid[2] and a number of other acids, particularly palmitic[3,4], are extracted along with the pigment. Prodigiosin probably occurs primarily as the salt of a fatty acid[4] in close association with the lipid portion of cell membranes[5]. Solutions of prodigiosin fluoresce when illuminated at 366 mμ, but deeply pigmented *Serratia* colonies do not[6], implying that within the cell the pigment is in bound form, possibly inaccessible to the aqueous ionic environment of the medium. The multiplicity of fractions in crude pigment extracts[4,7] may be partially the result of association of prodigiosin with different organic acids and partially of other natural pigments and artefacts. When prodigiosin itself is chromatographed, especially after exposure to light, acid, alkali or oxidants, additional coloured bands may appear. Such degradation products do not give rise to prodigiosin again on redevelopment. In a number of chromatographic systems, however, the orange free base form and the red protonated form of prodigiosin move as separate bands interconvertible by change of *p*H.

Because of the high absorptivity of prodigiosin in the visible region (at 537 mμ, $\varepsilon_{max} = 11{\cdot}2 \times 10^4$ in acidified 95 per cent ethanol)[4], solutions or chromatographic bands can be highly coloured and yet contain very small amounts of pigment. Consequently, minute traces of acid or base in adsorbents or solvents can produce detectable colour changes. Merely shaking an aqueous acetone or alcoholic solution of the orange basic form of prodigiosin will change the colour to red because of carbon dioxide picked up from the air[8].

We have carried out spectrophotometric titration of prodigiosin in approximately 44 per cent aqueous ethanol at 25° C using very dilute solutions of pigment to minimize any effects of association. Prodigiosin was extracted from disrupted *S. marcescens* cells, purified by solvent passage through the acid form and chromatography as the free base, and stored away from light as the free base. Immediately before titration, the sample was dissolved in chloroform and the solution washed with aqueous sodium hydroxide. The chloroform was evaporated and the

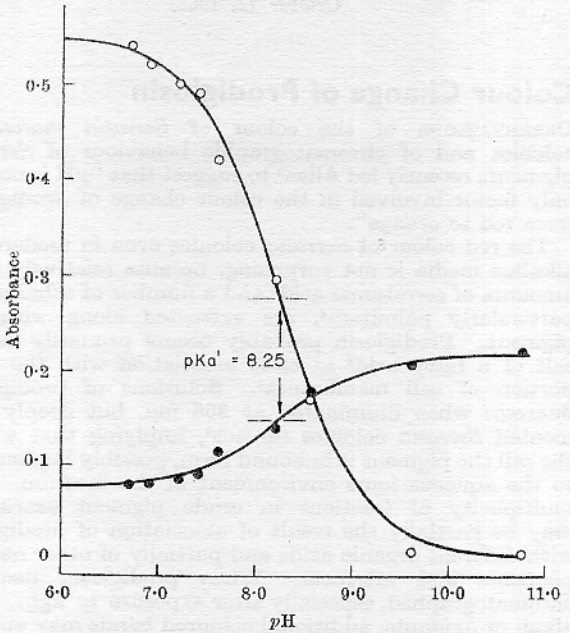


Fig. 1. Spectrophotometric titration of prodigiosin in approximately 44 per cent ethanol. ○, 537 mμ; ●, 470 mμ; solid lines calculated for a *p*H indicator of $pK_a' = 8{\cdot}25$.

residue dissolved in ligroin (boiling point 65°–67°; 'Skellysolve *B*', Skelly Oil Co.) for chromatography on a 12 cm column of diatomaceous earth ('Hyflo Supercel', Johns Manville Co.) developed with 0·25 per cent methanol in ligroin. The principal (orange) band initially eluted was collected and the solvent evaporated. The prodigiosin residue was dissolved in 95 per cent ethanol and samples (1 ml.) were added to tubes containing 4 ml. of aqueous buffer plus 2 ml. of 95 per cent ethanol. Buffers were made up to approximate *p*H values according to tables[9] and the *p*H was determined on the final solution with a Beckman model *G-S* *p*H meter and a Beckman glass electrode. Absorbance was determined against an alcohol–water blank with a Beckman *DB* spectrophotometer.

Fig. 1 shows that theoretical curves for an acid with $pK_a' = 8{\cdot}25$ fit the absorbance data for both the acidic maximum (537 mμ) and the alkaline maximum (470 mμ) of prodigiosin. Buffers used to cover the range of *p*H in Fig. 1 were phthalate, phosphate, *tris*(hydroxymethyl)-aminomethane and borate. We have similarly determined $pK_a'$ for prodigiosin in 50 per cent aqueous dioxane, using McIlvaine's phosphate-citrate buffers[10] over the *p*H range 5·5–9·5, obtaining a value of 7·65 from both curves. Stefanye[8] reported $pK_a'$ of 7·51 for prodigiosin

in aqueous acetone, using Sorensen (phosphate) buffers. The apparent acid dissociation constant, $K_a'$, of a *p*H indicator varies with the solvent and with solvent concentration for a particular solvent–water system[11].

Allen[1] interpreted her results with crude pigment extracts as indicating that prodigiosin "may exist in an oxidized (yellow) state or a reduced (red) state". We regard this interpretation as unnecessary and incorrect. The change of *p*H she observed in acetone solutions of prodigiosin, interpreted as evidence for oxidation of the pigment by acetone, may have been the result of dehydrating effects of the solution on the glass electrode[12]. The colour change she observed with a strong oxidizing agent like permanganate provides no basis for postulating that prodigiosin may be a respiratory co-factor. We see no evidence for an easily reversible oxidation-reduction reaction of prodigiosin or for a respiratory role of the pigment in the metabolism of *Serratia marcescens*[13].

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WALTER R. HEARN
JORGE MEDINA-CASTRO
MICHAEL K. ELSON

Department of Biochemistry and Biophysics,
Iowa State University,
Ames, Iowa.

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