Effects of Ionic Strength and pH on the Visible Absorption Spectrum of Crustacyanin from *Homarus vulgaris**

LIS WAHLGREN-BRÄNNSTRÖM and MARGARETA BALTSCHEFFSKY

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

A red shift in the absorption bands of carotenoids from several species of purple photosynthetic bacteria is obtained by illumination or by inducing a diffusion potential by K^+ in the presence of valinomycin. In some instances it may also be obtained in the dark by energization with ATP or PP_i.

Since the molecular mechanism underlying this carotenoid band shift has been widely discussed and it has been suggested that the shift is wholly due to a membrane potential [1] or partly due to conformational changes in the membrane proteins closely associated with the carotenoids,[2] it seemed to be of interest to study the influence of ionic strength and pH on a water soluble carotenoprotein. Crustacyanin, a carotenoprotein which contains astaxanthin as prosthetic group, was chosen for this purpose.

Crustacyanin has been studied by several groups, and extensive work has been done in an attempt to elucidate the mechanism behind the band shifts. These have been induced in various ways, such as addition of denaturing agents and changes in pH [3] or changes in the ionic strength [4] of the solution.

Experimental. Living lobsters, *Homarus vulgaris*, were obtained from the Swedish west coast. The carotenoprotein from lobster shell was isolated and partially purified by extraction with 10 % EDTA and ammonium sulfate precipitation, a modification of the method used by Cheesman *et al.*[4] The precipitate was dissolved in 0.05 M potassium phosphate buffer and dialyzed against 6 mM ammonium acetate. This material was then used in the experiments. A Cary 17 spectrophotometer was employed for recording the spectra.

In 0.05 M potassium phosphate buffer, pH 7, the sample showed three to four major peaks; at 280, 320, 410 and 630 nm. The peak at 410 nm was not present in all preparations and has been ascribed to the presence of a separate yellow protein.[5] After dialysis against 6 mM ammonium acetate, pH 6.6, the largest peak was shifted from 630 nm to 615 – 620 nm. This shift could be reversed by addition of potassium chloride, Fig. 1. Dialyzing the sample reversed the maximum back to 615 nm and again adding potassium chloride caused a gradual shift to 629 nm, Fig. 2. The same result was obtained with a previously frozen sample.

Three samples with the ionic strength of 0.006, 0.026 and 0.084 with the absorption maxima of 615, 625 and 628 nm, respectively, were passed through three Sephadex G 200 columns equilibrated at the same ionic strengths. Visual inspection of the columns revealed two bands, one blue in colour and one purple. The two bands could be seen in the column with an ionic strength of 0.006. A decrease in the amount of the purple band was observed when the ionic strength was raised, so that it was weaker in the column where the ionic strength

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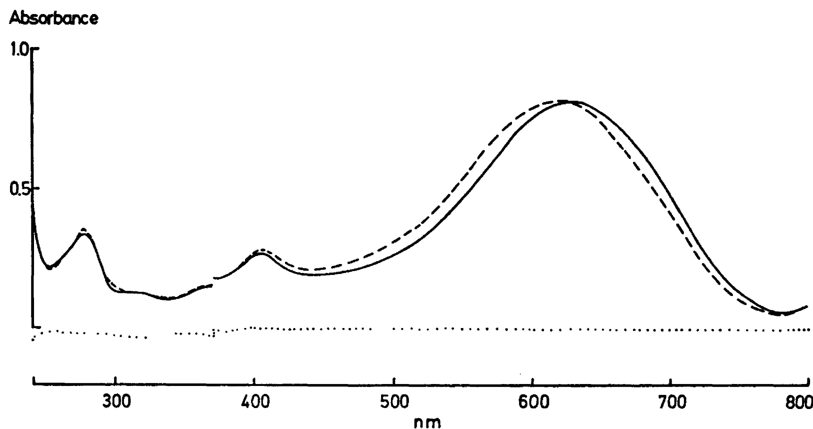

Fig. 1. The effect of changes in the ionic strength on the absorption spectrum. --- sample in 0.006 M ammonium acetate (after dialysis) $I = 0.006$ M —— potassium chloride added to give a final ionic strength of 0.15 M.

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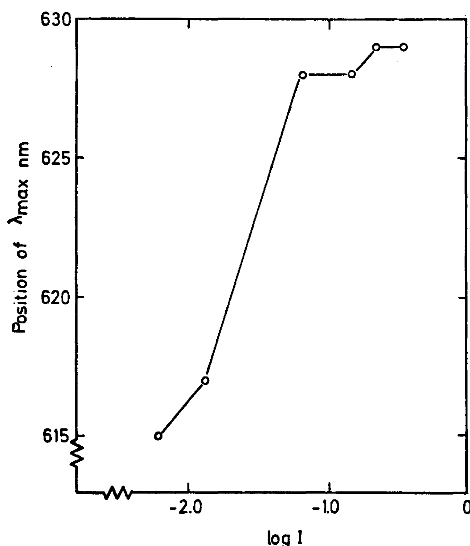

Fig. 2. The effect of changes in the ionic strength on the position of λ_{max}. The sample had previously been in high ($I = 0.15$ M) ionic strength before it was dialyzed against 0.006 M ammonium acetate. The ionic strength was then raised by successive additions of potassium chloride as shown.

was 0.026 and it could not be seen by the eye in the column with an ionic strength of 0.084.

The two fractions were collected from each column: the blue one had a λ_{max} at 630 nm and a molecular weight larger than 240 000, and the purple fraction had a λ_{max} at 590 nm and a molecular weight of approximately 50 000 which possibly could be the β-form previously described by Cheesman *et al.*[4] When these two fractions were mixed in different proportions intermediate absorption maxima were achieved.

The appearance of the visible spectrum as a function of pH was found to be influenced also by the ionic strength: (i) At low ionic strength (dialyzed sample) the peak could be reversibly moved to lower wavelengths by raising the pH of the sample, (ii) at high ionic strength (which had been achieved by addition of potassium chloride) the peak remained at 630 nm when the pH was being raised, but could be reversibly shifted to lower wavelengths upon acidification of the sample.

Cheesman *et al.*[4] also noted what supposedly was a gradual shift in the absorption induced by a change in ionic strength. Their studies were, however, performed at a fixed wavelength, the whole spectrum was only studied at the extreme conditions (*i.e.* when the sample was in 0.2 M phosphate buffer, pH 7, and after dialysis against distilled water).

By studying the whole spectrum we found that the large band shifts were not accompanied by any change in the maximum extinction.

The spectral shifts described here appear as true electrochromic band shifts [6] and may be due to a redistribution of charges close to or at the chromophore binding site(s) upon aggregation and deaggregation of the protein.

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