Evidence of Association of Glucagon from Optical Rotatory Dispersion and Concentration-Difference Spectra

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The ready crystallizability of glucagon from alkaline solutions (Staub, Sinn, and Behrens, 1955) contrasts sharply with the observation by Kay and Marsh (1959) that glucagon exists as a random coil in glycine buffer, pH 10.4, i.e., under conditions not differing widely from the crystallization conditions. It would seem that definite molecular conformations are a prerequisite for crystallization of any chain-molecular substance. One of us has presented X-ray diffraction evidence (King, 1959, 1965) lending weight to the idea that glucagon is α-helical in the crystal. We shall present evidence from optical rotatory dispersion and concentration-difference spectra that glucagon molecules transform from an associated, helical form existing in solution at concentrations approaching saturation, to a dissociated, random-coil form upon dilution.

EXPERIMENTAL

The material used in these studies was porcine pancreatic glucagon from the Lilly Research Laboratories. All solutions were prepared in 0.2 M potassium phosphate buffers. The glucagon was dissolved by warming to 60°

in a buffer giving the desired final pH, and then cooling to the temperature of measurement. Dilutions were made by adding a buffer of the same pH. The solutions were filtered through sintered glass.

Optical rotations were measured in the Perkin-Elmer 141 Polarimeter at pH 10.0 at 40°. This temperature was chosen to permit attaining glucagon concentrations up to ~1.3%, since glucagon crystallizes out of these solutions at room temperature. The data (generally for 365, 436, 546, and 589 mμ) were fitted to a one-term Drude equation

$$[\alpha]_\lambda = \underline{A}/(\lambda^2 - \lambda_c^2)$$

by a least-squares method described by Schellman (1958).

The concentration-difference spectra were measured by the method of Fisher and Cross (1965) in the Zeiss MK3 Spectrophotometer at room temperature at pH values of 8 and 10. The initial glucagon concentrations were 0.3-0.4%, and the concentration-difference spectra were measured after tenfold and fiftyfold dilutions.

RESULTS

Fig. 1 shows the values of the parameters λ_c and $\underline{A}$ plotted against the concentration $\underline{c}$, together with the unweighted regression lines for λ_c and $\underline{A}$. The values of λ_c rise from about 225 mμ to 260 mμ over the studied concentration range, with a concomitant fall in $\underline{A}$. Thus, λ_c ranges with increasing concentration from a value typical of denatured and other non-helical proteins to a value characteristic of highly helical proteins (see Urnes and Doty, 1961).

The concentration-difference spectra in the 250-320 mμ range showed Beer's Law deviations at both pH 8 and 10. The bulk of the spectral change occurred in the dilution from the original concentration

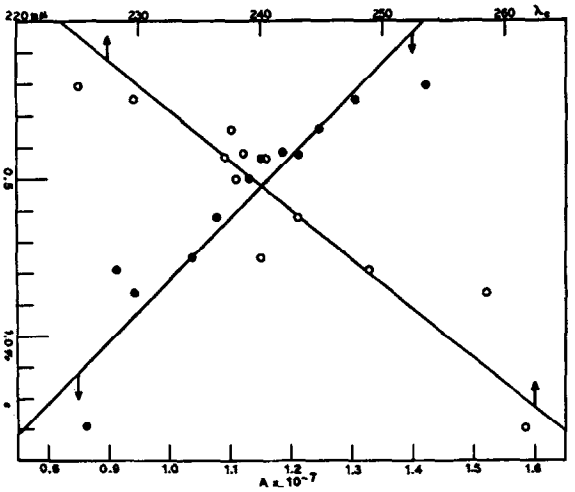

Fig. 1. Variation of the Drude-equation parameters λ_c (o) and $\underline{A}$ (•) as functions of the concentration $\underline{c}$.

$\underline{c}$ to 0.1 $\underline{c}$. The difference curves for the dilution from 0.1 $\underline{c}$ to 0.02 $\underline{c}$ were essentially featureless. This, and the fact that the curves showed essentially the same features in separate runs subject to independent dilution errors, indicated that the observed difference spectra are not artifacts arising from dilution errors.

Fig. 2 shows the values of $\Delta\underline{E} = \underline{E}_{0.02\ \underline{c}} - \underline{E}_{\underline{c}}$ for two typical runs. Other runs at pH 10 showed essentially the same features as the lower curve.

The minima in these curves in the 293-295 mμ region can be reasonably ascribed to exposure of the tryptophan residue to solvent upon dilution, while the minima in the 285-290 mμ region can be similarly ascribed to tyrosine. This evidence would suggest that glucagon exists at higher concentrations in a form in which both tyrosine and tryptophan are involved in hydrophobic regions, but these residues are exposed to solvent upon dilution.

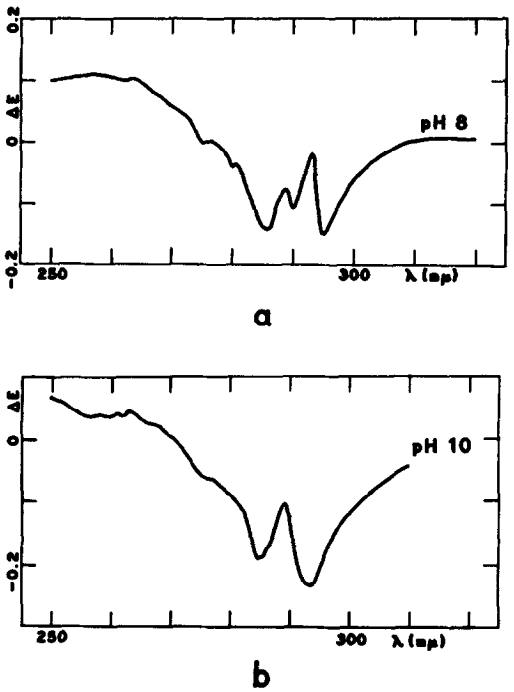

Fig. 2. Concentration-difference spectra of glucagon.

a) pH = 7.97 ± 0.07 (maximum deviation), $\underline{c}$ = 3.49 g/l glucagon.

b) pH = 9.94 ± 0.02, $\underline{c}$ = 3.42 g/l.

DISCUSSION

If we assume that the mechanism of the reaction accompanying dilution does not differ for the two temperatures studied, the combination of evidence from optical rotatory dispersion and concentration-difference spectra suggests that glucagon dissociates upon dilution from a helical, associated form existing at concentrations near saturation, to a non-helical, dissociated form.

These results can be interpreted on the basis of a hypothetical model of glucagon aggregates in solution closely resembling the triads of helices postulated to exist in the crystal. We assume that α-helical

glucagon molecules are labile in isolation, but are stabilized in certain aggregates, and that the predominant forces inducing aggregation are hydrophobic. This view is favored by our observation that glucagon crystals are stabilized by electrolytes, which minimize electrostatic forces by shielding, but are destroyed by small amounts of organic solvents (e.g., 20% methanol in the medium), which would decrease the hydrophobic interactions.

Fig. 3a shows the distribution of hydrophobic residues in an α-helical model of glucagon. These residues occupy two regions on opposite sides at opposite ends of the rodlike molecule. Manipulation of such models to fit the hydrophobic "grease spots" to one another shows that contact cannot be maximized well when handling only two molecules. However, three molecules lend themselves to a packing that does this in the manner shown

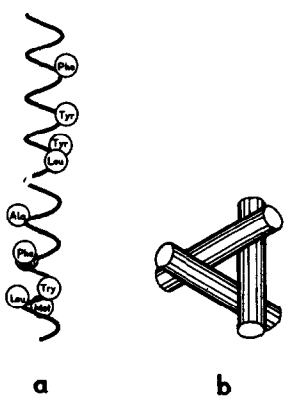

Fig. 3. a) Diagram showing the concentration of hydrophobic residues in two specific areas in the α-helical model of glucagon.

b) Packing of three rodlike molecules to maximize hydrophobic contacts (cf. King, 1965, Fig. 3).

in Fig. 3b, with the molecules arranged about a threefold symmetry axis. Similar triads of molecules are found in the crystal, suggesting that the trimer may well be the common feature shared by the crystals and by their concentrated solutions. We are undertaking sedimentation studies to determine whether this prediction is borne out.

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REFERENCES

Fisher, H.F., and Cross, D.G. (1965). Arch. Biochem. Biophys. 110, 217.

Kay, C.M., and Marsh, M.M. (1959). Biochim. Biophys. Acta 33, 251.

King, M.V. (1959). J. Mol. Biol. 1, 375.

King, M.V. (1965). J. Mol. Biol. 11, 549.

Schellman, J.A. (1958). Compt. rend. trav. lab. Carlsberg, Sér. chim. 30, 363.

Staub, A., Sinn, L., and Behrens, O.K. (1955). J. Biol. Chem. 214, 619.

Urnes, P., and Doty, P. (1961). Advances in Protein Chemistry 16, 401.