The solubility of proteins in the presence of carbon dioxide

In a previous communication[1], we reported the fractionation of proteins with a cellulose-derived anion exchanger and a system of water and CO_2. We have now observed that CO_2 increases the rate of solution of proteins in water, and in some cases renders insoluble proteins soluble.

The increase in rate of solution is especially valuable for making concentrated solutions of bulky solids. In the preparation of a 30% solution of serum albumin, a round-bottomed flask was filled with CO_2, albumin powder (Cohn Fraction V) was added, then water, and the flask was stoppered and placed in the cold. Within 2 h solution was complete, whereas a control without CO_2 still contained most of its solid phase. No precipitate was obtained on removal of the CO_2. 2% solutions of haemoglobin, which under ordinary conditions require grinding and careful stirring, can be prepared simply by adding the freeze-dried powder to distilled water and covering with an atmosphere of CO_2. Dilute acetic acid could not duplicate the effect of the CO_2 on the solubilization of these soluble proteins.

CO_2-treatment is a useful general method for solubilizing some insoluble proteins under mild conditions. The technique has application in chromatographic procedures. In one experiment, 200 mg porcine serum γ-globulin (Cohn Fraction II) on a 5 g aminocellulose column (free-base form) was eluted with two hold-up volumes of distilled water saturated with CO_2 (1 atm.)[1]. A voluminous precipitate which formed almost immediately as the protein fractions were being collected was attributed to the escape of CO_2. Confirmatory experiments demonstrated that water-clear, 10% solutions of bovine or porcine serum fraction II can be prepared in distilled water under an atmosphere of CO_2. Agitation, which may be a source of denaturation, is not required. Replacement of the CO_2 with air causes the reprecipitation of much of the protein. Mild denaturation renders porcine γ-globulin insoluble in CO_2-water. In one case withdrawal of CO_2 resulted in crystallization of a protein not crystallized heretofore in this laboratory. A kidney tissue preparation desorbed with CO_2 from the aminocellulose (fraction 43 in the previous communication[1]), yielded crystals on standing unstoppered in the cold. The crystalline precipitate, amounting to almost one-half the total protein present, was easily separated. Upon exposure to CO_2 at one atmosphere the crystals quickly and completely redissolved. On withdrawal of the atmosphere of CO_2, the protein began to reprecipitate (requiring several days for completion). The crystals were also soluble in dilute salt (0.1 *M*) and did not reprecipitate on dilution with water. Crystalline zinc insulin was solubilized to a small but definite amount (0.1 mg/ml) by treatment with CO_2. Removal of the CO_2 atmosphere over the insulin solutions caused a precipitate to form starting at the liquid–gas interface. A euglobulin fraction made from bovine serum by the method of GREEN[2], or from pancreas by a similar procedure, was not solubilized. The latter material could be solubilized with dilute acetic acid.

Chemical changes due to interaction of CO_2 with proteins[3] have not been observed by the usual physical methods. Release of the CO_2 from these treated solutions has no apparent effect on the soluble proteins but causes the precipitation of the less soluble. After treatment with CO_2 no alteration is noted in the solubility characteristics of crystalline egg lysozyme, in the electrophoretic behavior of serum albumin, or in the absorption spectrum of γ-globulin. These observations indicate that the CO_2 which hydrates and ionizes to a very small degree in distilled water has a very great and reversible effect on the solubility of proteins in water.

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[1] M. A. MITZ AND S. S. YANARI, *J. Am. Chem. Soc.*, 78 (1956) 2649.
[2] A. A. GREEN, *ibid.*, 60 (1938) 1108.
[3] W. C. STADIE AND H. O'BRIEN, *J. Biol. Chem.*, 117 (1937) 439.

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Swelling of mitochondria isolated from different tissues

In a previous report[1], it was demonstrated that the swelling of mitochondria produced *in vitro* by thyroxine and its analogues varies according to the tissue from which the mitochondria are isolated. Thus thyroxine causes pronounced swelling of rat liver and kidney mitochondria, slight swelling of those from diaphragm and heart, and essentially none with mitochondria isolated from spleen, brain, or testis. This communication demonstrates that mitochondria from these tissues vary also in their response to other agents which have previously been shown to cause swelling of isolated liver mitochondria[2]. The results serve to emphasize that data obtained with mitochondria isolated from any particular tissue must be interpreted in terms of that tissue only.

Mitochondria were isolated[1] from the organs of normal adult male Wistar rats, suspended

in a medium containing 0.3 *M* sucrose and 0.02 *M* tris(hydroxymethyl)aminomethane, pH 7.4, and the degree of swelling produced by the various compounds measured spectrophotometrically as previously described[2]. Results are expressed as the minimum concentration of added compound required to produce a 15–25% increase in swelling in 30 min. It is apparent from Table I that the swelling produced by any given compound varies markedly with mitochondria from different tissues. The sensitivity of liver mitochondria to calcium and orthophosphate is much greater than that of mitochondria from any other tissue tested; spleen mitochondria, on the other hand, are remarkably stable to all compounds except digitonin, to which they are unusually sensitive. It is of interest to note that although mitochondria isolated from spleen, brain, and testis are insensitive to the swelling effect of thyroxine[1], they undergo swelling in the presence of low concentrations of digitonin.

TABLE I

SWELLING OF MITOCHONDRIA FROM DIFFERENT RAT TISSUES*

Tissue	Concentration of added compound (*M*)					
	Digitonin	*$CaCl_2$*	*Orthophosphate*	*Pentachlorophenol*	*p-Chloromercuribenzoate*	*$HgCl_2$*
Liver	$4 \cdot 10^{-5}$	$2 \cdot 10^{-5}$	$2 \cdot 10^{-3}$	$4 \cdot 10^{-4}$	$2 \cdot 10^{-5}$	$3 \cdot 10^{-6}$
Kidney	$8 \cdot 10^{-5}$	$2 \cdot 10^{-3}$	$1 \cdot 10^{-1}$	$4 \cdot 10^{-4}$	$2 \cdot 10^{-5}$	$3 \cdot 10^{-6}$
Heart	$1.6 \cdot 10^{-4}$	$1 \cdot 10^{-3}$	$1 \cdot 10^{-2}$	$4 \cdot 10^{-4}$	$4 \cdot 10^{-5}$	No effect in $4 \cdot 10^{-3}$
Spleen	$2 \cdot 10^{-6}$	No effect in $5 \cdot 10^{-2}$	$1 \cdot 10^{-1}$	$4 \cdot 10^{-3}$	No effect in $5 \cdot 10^{-3}$	No effect in $4 \cdot 10^{-3}$
Brain	$4 \cdot 10^{-5}$	–	–			–
Testis	$4 \cdot 10^{-5}$	–	–	$1 \cdot 10^{-3}$		–
Diaphragm	$1.6 \cdot 10^{-4}$	–	$4 \cdot 10^{-2}$	$4 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	–

* Results are expressed as the minimum concentration required to give in 30 min a 15%–25% increase in swelling over a control suspension in 0.3 *M* sucrose and 0.02 *M* tris(hydroxymethyl)-aminomethane, pH 7.4.

Considerable variability has been observed with preparations of liver mitochondria from different animals[1,2] even though isolation procedures have been rigidly standardized. Preliminary studies suggest that such variability may be related, at least in part, to the nutritional status of the animal. Thus it may be seen in Table II that the response to added thyroxine and digitonin s much greater in mitochondria from normal livers than in those from fasted animals.

TABLE II

EFFECT OF FASTING ON MITOCHONDRIAL SWELLING*

	$3 \cdot 10^{-6}$ *M* L-*Thyroxine*	$4 \cdot 10^{-5}$ *M* *Digitonin*
Normal liver	28	28
Fasted liver (24 h)	4	4

* Each value represents the percentage decrease in optical density of a suspension of mitochondria containing the compound tested minus that observed in a control sample containing only 0.3 *M* sucrose and 0.02 *M* tris(hydroxymethyl)aminomethane, pH 7.4. These values are taken as a measure of the swelling produced by the compound. Control swelling was identical in the two preparations.

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[2] D. F. TAPLEY, *J. Biol. Chem.*, 222 (1956) 325.

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