in animal enzyme preparations. It may be noted that the β-oxidation of propionate to malonyl-CoA would provide a means for the formation of the latter compound, an intermediate in fatty acid synthesis, other than by acetyl-CoA carboxylation[12–15].

This work was aided by Grant No. A-739 from the U.S. Public Health Service (to J.R.S.) and a grant from the National Science Foundation (to M.J.C.).

Department of Pharmacology, School of Medicine, Western Reserve University, Cleveland, Ohio (U.S.A.) — GOVIND K. K. MENON, JOSEPH R. STERN

Department of Biological Chemistry, Medical School, University of Michigan, Ann Arbor, Mich. (U.S.A.) — FLOYD P. KUPIECKI, MINOR J. COON

[1] G. RENDINA AND M. J. COON, *J. Biol. Chem.*, 225 (1957) 523.
[2] H. DEN, W. G. ROBINSON AND M. J. COON, *J. Biol. Chem.*, 234 (1959) 1666.
[3] E. ROBERTS AND H. M. BREGOFF, *J. Biol. Chem.*, 201 (1953) 393.
[4] F. P. KUPIECKI AND M. J. COON, *J. Biol. Chem.*, 229 (1957) 743.
[5] J. GIOVANELLI AND P. K. STUMPF, *J. Biol. Chem.*, 231 (1958) 411.
[6] P. R. VAGELOS AND J. M. EARL, *J. Biol. Chem.*, 234 (1959) 2272.
[7] J. R. STERN, M. J. COON, A. DEL CAMPILLO AND M. C. SCHNEIDER, *J. Biol. Chem.*, 221 (1956) 15.
[8] G. K. K. MENON, AND J. R. STERN, *Federation Proc.*, 18 (1959) 287.
[9] J. R. STERN, *J. Biol. Chem.*, 221 (1955) 33.
[10] J. R. STERN, *Biochim. Biophys. Acta*, 26 (1957) 448.
[11] P. R. VAGELOS, *J. Biol. Chem.*, 235 (1960) 346.
[12] S. J. WAKIL, *J. Am. Chem. Soc.*, 80 (1958) 6465.
[13] R. O. BRADY, *Proc. Nat. Acad. Sci. U.S.*, 44 (1958) 993.
[14] J. V. FORMICA AND R. O. BRADY, *J. Am. Chem. Soc.*, 81 (1959) 752.
[15] S. J. WAKIL AND J. GANGULY, *J. Am. Chem. Soc.*, 81 (1960) 2597.

Received August 15th, 1960

Biochim. Biophys. Acta, 44 (1960) 602–604

Dissociation of pyruvic kinase in urea solutions

Crystalline pyruvic kinase prepared from rabbit muscle according to the method for "fluorokinase" of TIETZ AND OCHOA[1], dissociates in concentrated urea solutions into smaller fragments. In 2.5–3 M urea both forms (undissociated and dissociation products) are clearly separated in the ultracentrifuge (Fig. 1).

The sedimentation coefficient ($S_{20,w}$) of the undissociated form drops from 10.4 S in phosphate buffer (pH 6), to 7.5 S in 3 M urea, as a result of an increase in the friction coefficient. In 6 M urea the dissociation is complete, and the product sediments in the ultracentrifuge as a single, fairly symmetrical peak (Fig. 2). Its sedimentation coefficient, determined at 0.6% protein concentration in 6 M urea (dissolved in phosphate buffer, pH 6, I 0.1, for the elimination of charge effects) amounts to 2.2 S.

The diffusion coefficient ($D_{20,w}$) determined under the same conditions is $1.4 \cdot 10^{-7}$ cm^2/sec.

The molecular weight of the dissociation product calculated from the above values is 150,000, assuming the partial specific volume to be the same as for the intact enzyme, viz. 0.75 cm^3/g^2. The friction coefficient (f/f_0) is 4.2. Native pyruvic kinase has a molecular weight within the range 230,000–237,000[2,3], so it seems that its molecule is split by strong urea into two parts. Since molecular-weight determinations in

concentrated urea are uncertain[4], one cannot exclude the possibility that these fragments may be the halves of the original molecule.

Similar dissociation was observed at pH 12 in glycine buffer, but the dissociation products showed marked inhomogeneity in the ultracentrifuge.

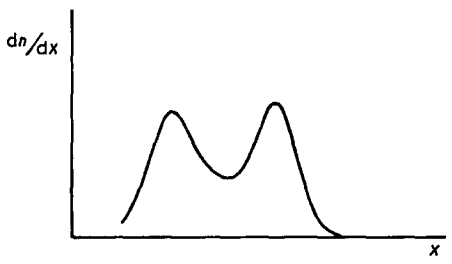

Fig. 1. Lamm scale sedimentation diagram of pyruvic kinase in 3 *M* urea after 60 min at 50,000 rev./min (184,000 × *g*). Protein concentration, 0.6 g/100 ml. Sedimentation from left to right.

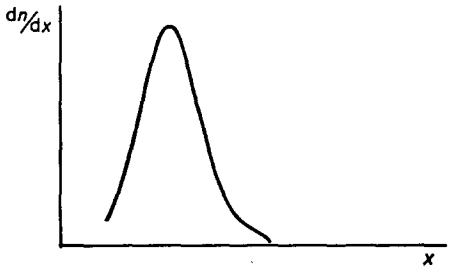

Fig. 2. Lamm scale sedimentation diagram of pyruvic kinase in 6 *M* urea (dissolved in phosphate buffer, pH 6, I, 0.1) after 180 min at 50,000 rev./min. Protein concentration, 0.6 g/100 ml.

The enzymic activity of pyruvic kinase diminishes in the presence of urea, and at 2.5 *M* it completely disappears. About 80 % of the activity can then be recovered by the removal of the urea, *e.g.* by dialysis. The irreversibly inactivated fraction is insoluble in neutral salt solutions of I, 0.1.

Inactivation by 5 *M* urea is mostly irreversible. After slow removal of the urea, about 80 % of the protein becomes insoluble in 0.1 *M* phosphate buffer (pH 6). The remaining protein shows only approx. 5 % of the total original activity.

After incubation with 0.1 *M* thioglycolate for 48 h at 2°, the activity of the pyruvic kinase is diminished to approx. 15 %. However, the sedimentation coefficient is not influenced.

These experiments show that the pyruvic kinase molecule consists of at least two polypeptide chains joined together by means of hydrogen bonds.

Institute of Immunology and Experimental Therapy, Biochemistry Department, Wroclaw (Poland) A. MORAWIECKI

[1] A. TIETZ AND S. OCHOA, *Arch. Biochem. Biophys.*, 78 (1958) 477.
[2] R. C. WARNER, *Arch. Biochem. Biophys.*, 78 (1958) 494.
[3] A. MORAWIECKI, *Acta Biochim. Polon.*, 5 (1958) 437.
[4] K. SCHACHMAN, *Ultracentrifugation in Biochemistry*, Academic Press, New York, 1959, p. 82.

Received September 2nh, 1960