possibility, which would imply specificity of binding between messenger RNA and ribosomes, is presently being explored.

A somewhat analogous situation is found in a system derived from unfertilized sea urchin eggs. Monroy, Maggio, and Rinaldi (20) have reported that this system responds to poly U but not to RNA obtained from the same source or from sea urchin embryos. They present evidence that indicates the existence of a ribosome-bound protein that would interfere with the binding of the native messenger RNA to the particles and that can be destroyed by trypsin digestion.

Treatment of ungerminated wheat germ ribosomes with different amounts of trypsin did not significantly activate either the endogenous amino acid incorporation or the polymerization of proline in the presence of poly C. Ribosomes obtained from wheat germ imbibed during 24 hours at room temperature did not bind proyl-sRNA when incubated with poly C.

The evidence accumulated from different species leaves little doubt that the genetic code is essentially universal, despite the fact that the components involved in protein synthesis have mutated throughout evolution. As discussed by Crick (21), a change in the code would involve a mutation affecting the translation mechanism which rests upon the specificity of the aminoacyl-RNA synthetases. Unless this mutation would affect a very infrequently used triplet, it would be lethal since it would alter the primary structure of most cellular proteins. These considerations imply that all presently living species arose from a common ancestor which had the same degenerate code that is presently being unrefereed.

Acknowledgment—It is a pleasure to acknowledge the skillful technical assistance of Miss Lucinda Nunez.

REFERENCES


Separation of H⁺ and OH⁻ in the Extramitochondrial and Mitochondrial Phases during Ca⁺⁺-activated Electron Transport* (Received for publication, January 6, 1966)

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When electron transport in isolated mitochondria is stimulated by divalent ions such as Ca⁺⁺ (1-5) or monovalent ions such as K⁺ in the presence of valinomycin or gramicidin (6, 7), H⁺ ions are ejected into the medium. During Ca⁺⁺-stimulated respiration, about 2.0 H⁺ ions appear and 2 Ca⁺⁺ ions are accumulated as each pair of electrons passes each energy-conserving “site” in systems at pH near 7.4 and salt concentrations near 80 mM (1-5, 8-12). These findings have been taken as support for the view that electron transport can produce a pH gradient across the mitochondrial membrane (7, 13-15). However, little quantitative information is available on acid-base changes occurring within intact mitochondria during electron transport.

This communication reports experiments on the acid-base changes in the mitochondrial phase that accompany the appearance of H⁺ ions in the medium during Ca⁺⁺-stimulated oxygen uptake. From pH measurements and acid-base titrations on rat liver mitochondria “solublized” in a nonionic detergent before and after Ca⁺⁺-induced respiratory jumps, it has been found that the mitochondrial phase loses H⁺ ions in an amount that is nearly exactly equal to the H⁺ appearing in the suspending medium. This finding thus provides more complete evidence that the respiratory chain may act as a directional “H⁺ pump.”

Fresly prepared rat liver mitochondria were added to a medium of 80 mM NaCl and 5 mM sodium succinate, lightly buffered with either Tris-chloride or glycyglycine; the respiratory jump was initiated by addition of 4CaCl₂. Aliquots of the mitochondria were taken before and after Ca⁺⁺ addition, rapidly chilled, and centrifuged at 10,000 x g. Uptake of 4Ca⁺⁺ from the medium was measured as described before (8). H⁺ ejection was measured with a glass electrode and a Beckman Expandomatic pH meter coupled to a Sargent SR recorder. The absolute amounts of H⁺ ejected were determined by comparison with pH changes yielded on addition of internal standards of HCl in each experiment. Oxygen uptake was measured with the Clark oxygen electrode.

The measurement of acid-base changes occurring in the mitochondrial phase during Ca⁺⁺-induced respiratory jumps was developed from Mitchell’s finding that nonionic detergents abolish the impermeability of the mitochondrial membrane to H⁺ (cf. References 13 and 14). The mitochondrial pellets obtained from 2.0 ml of the suspension before Ca⁺⁺ addition and 30 sec after completion of the Ca⁺⁺-induced respiratory jump were carefully dried of adhering medium and taken up in 2.0 ml of a neutral solution of Lubrol (a nonionic detergent; ICL Organics, Inc., Providence, Rhode Island) in water (40 µg per ml). All of the mitochondrial pellet “dissolved” to yield a

* This investigation was supported by Grant GM 05919 to A. L. L. and Grant Tw 00115 to C. S. R. from the National Institutes of Health and a grant to A. L. L. from the National Science Foundation.
nearly clear solution; no material sedimented from such solutions on standing. The pH of the solutions of the mitochondrial pellets was then determined with the glass electrode. The Lubrol solution of the mitochondrial pellet collected before the addition of Ca++ was then supplemented with an amount of CaCl₂ equivalent to the amount of Ca++ actively taken up during the jump, as determined by actual measurement of ΔCa++ uptake, so that the "before" and "after" mitochondrial solutions contained the same amount of Ca++. The addition of such amounts of CaCl₂ decreased the pH of the before solution by about 0.1 pH, as might be expected if the mitochondria contain anions capable of binding Ca++. The before solution was then titrated with known increments of standard NaOH over a range of pH extending to and beyond the observed pH of the after extract. Fig. 1 shows the titration curve for the before pellet of Experiment 1 of Table I. The net loss of H⁺ (or gain of OH⁻) that occurred in the mitochondrial phase during the Ca++ jump could then be determined from the titration curve which was linear over the pH range of 6.7 to 8.1. In the experiment of Fig. 1, the solubilized mitochondria before the Ca++-induced respiratory jump had a pH of 6.74 (with Ca++ added) and after the jump of 7.58. From the titration curve this increase in pH corresponded to a gain of 360 mpmoles of OH⁻ in the mitochondrial phase. The validity of the method was checked by back-titration (with standard HCl) of the solution of Ca++-loaded mitochondria collected after the Ca++ jump to the pH of the before solution; this curve is also shown in Fig. 1. The back-titration curve obtained coincided nearly exactly with the forward titration curve, showing that loading of mitochondria with Ca++ does not cause irreversible changes in the proton donor or acceptor groups titrating in the pH zone examined.

Table I shows data from several experiments on the initial and final pH of the suspending medium and of the solubilized mitochondrial phase, the absolute amounts of H⁺ formed in the external medium and of OH⁻ formed in the mitochondrial phase, as well as measurements of the Ca++ taken up. The observed molar ratio of H⁺ ejected to Ca++ accumulated was found to vary in Experiments 1 to 4 from 0.98 to 1.10 and the ratio of OH⁻ accumulated to Ca++ accumulated from 0.79 to 1.06. The molar ratio of H⁺ ejected to OH⁻ accumulated varied from 0.01 to 1.24 in the experiments in Table I. From all of the experiments carried out to date, the average of this ratio is about 0.97. The H⁺:OH⁻ ratio did not change significantly when the starting pH of the medium was varied over the range 6.90 to 8.0. No significant change in external or internal pH occurred in the absence of added Ca++ over a period of 3 min, or more than twice the time required for the complete respiratory jump induced by Ca++. These findings show that for each Ca++ ion accumulated during the respiratory jump, 1 H⁺ ion appears in the medium and 1 OH⁻ ion accumulates in the mitochondrial phase. Since 2.0 Ca++ ions are accumulated per pair of electrons per energy-conserving site in this system under the conditions used (1–5, 8–12), 2 H⁺ ions appear in the suspending medium and 2 OH⁻ ions accumulate within the mitochondrial phase per pair of electrons per site traversed. In effect, the passage of a pair of electrons along the complete respiratory chain can bring about the net transfer of 6 H⁺ ions from the mitochondrial phase to the extramitochondrial phase, in support of Mitchell's view (14). Additon of 2,4-dinitrophenol to the system prior to addition of Ca++ completely prevented both the formation of the pH gradient and the uptake of Ca++ (cf. Experiment 5 of Table I); antimycin A produced a similar effect. Similarly, the addition of dinitrophenol to a mitochondrial system after the production of a pH gradient in a Ca++-induced jump brought about imme-

![Fig. 1. Titration of the before pellet with NaOH and of the after pellet with HCl. Additions of acid or base were made with a microburette. Data are for 5.0 mg of mitochondrial protein.](image_url)
concluded on the basis of these data that protein polypeptide chains in concentrated guanidine hydrochloride are true random coils retaining no elements of their original native conformation. Intrinsic viscosity, [\eta], is one of the most sensitive measures of gross conformation of macromolecules. For spheres which do not interact with the solvent, [\eta] is very small and entirely independent of molecular weight (1). For compact, globular proteins in their native state, a result close to this is obtained (2). Rod-shaped particles and random coils, on the other hand, have much larger values of [\eta], and they increase with molecular weight (2-4). For long chains,

\[ [\eta] = K' n^a = K'(M_0)^a \]  

(1)

where \( n \) is the number of monomer units (in this case amino acid residues) per chain, \( M_0 \), the average molecular weight per monomer, and \( K' \), \( K'' \), and \( a \) are constants. Rods and random coils can be distinguished by the value of \( a \), which is about 1.8 for rods, but usually lies in the range of 0.5 to 0.8 for random coils.

For homopolymers, \( M_0 \) of Equation 1 can be, and usually is, replaced by the molecular weight, \( M \), but in proteins and polypeptides, which contain a variety of monomer units, \( M_0 \) can vary from one molecule to another, and \( n \) must be used as the variable indicative of molecular size. As a matter of fact, perfect adherence to Equation 1 should not be expected when different proteins and polypeptides are compared because the amino acid side chains are expected to influence the freedom of rotation about single bonds (5) as well as influencing weak long range interactions (see below).

The protein used in this study and the measured viscosities are shown in Table I. The solvent in each case was 6 M guanidine hydrochloride, containing 0.1 or 0.2 M \( \beta \)-mercaptoethanol, introduced so as to break disulfide bonds where present or to prevent their formation by slow oxidation of thiol groups. Identical results are obtained with or without mercaptoethanol for myoglobin, which contains neither cysteine nor cystine residues. The molecular weights and chain lengths listed in the table are those of the constituent polypeptide chains. The appropriate "viscosity average" has been used for those proteins which contain more than one kind of chain. Molecular weight determinations by sedimentation equilibrium were carried out for each protein (except insulin) to ascertain that dissociation to the ultimate polypeptide chains had actually occurred.

### Proteins in 6 M Guanidine Hydrochloride

#### DEMONSTRATION OF RANDOM COIL BEHAVIOR

(Received for publication, January 29, 1966)

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The purpose of this paper is to report measurements of the intrinsic viscosities of protein polypeptide chains in concentrated aqueous solutions of guanidine hydrochloride in the presence of \( \beta \)-mercaptoethanol to rupture disulfide bonds where they exist. The results show that the intrinsic viscosity depends on molecular weight exactly as predicted for randomly coiled polymer chains. The end-to-end distances calculated from the data are of the order of magnitude expected on the basis of the bond lengths and angles of the polypeptide chain, and are comparable with similar data for randomly coiled poly-\( \gamma \)-benzyl-l-glutamate. It is

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**Table I**

**Viscosities and dimensions of polypeptide chains of proteins in 6 M guanidine hydrochloride**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular weight</th>
<th>Residues per chain</th>
<th>([\eta] )</th>
<th>End-to-end distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>2,970</td>
<td>26</td>
<td>6.1</td>
<td>44</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>13,800</td>
<td>124</td>
<td>16.0</td>
<td>101</td>
</tr>
<tr>
<td>Homologenin</td>
<td>15,500</td>
<td>144</td>
<td>18.0</td>
<td>112</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>17,200</td>
<td>158</td>
<td>20.9</td>
<td>120</td>
</tr>
<tr>
<td>( \beta )-Lactoglobulin</td>
<td>18,400</td>
<td>162</td>
<td>22.8</td>
<td>126</td>
</tr>
<tr>
<td>Chymotrypsinogen</td>
<td>25,200</td>
<td>242</td>
<td>28.5</td>
<td>145</td>
</tr>
<tr>
<td>Glyceraldehyde 3-phosphate dehydrogenase*</td>
<td>36,300</td>
<td>331</td>
<td>34.5</td>
<td>181</td>
</tr>
<tr>
<td>Aldolase</td>
<td>40,000</td>
<td>365</td>
<td>35.5</td>
<td>189</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>69,000</td>
<td>627</td>
<td>52.2</td>
<td>258</td>
</tr>
<tr>
<td>Thyroglobulin*</td>
<td>115,000</td>
<td>1,500</td>
<td>82</td>
<td>401</td>
</tr>
<tr>
<td>Myosin*</td>
<td>197,000</td>
<td>1,790</td>
<td>92.6</td>
<td>443</td>
</tr>
</tbody>
</table>

* This work was supported by grants from the National Science Foundation and from the National Institutes of Health, United States Public Health Service.

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* Data in 5 M guanidine hydrochloride from Reference 6.

* Data in 5 M guanidine hydrochloride from References 7 and 8.