Studies on Amino Acyl Transfer from Soluble-RNA to Rat Liver Ribonucleoprotein Particles; Effect of Soluble and Microsomal Extracts*

JUNE M. FESSENDEN† AND KIVIE MOLDAVE

From the Department of Biochemistry, Tufts University School of Medicine, Boston, Massachusetts

Received December 12, 1961

The transfer of amino acid from amino acyl *soluble*-RNA to microsomal and ribosomal protein is catalyzed by the supernatant fraction of rat liver homogenates. A highly purified soluble factor has been obtained from the supernatant; this factor catalyzes amino acyl transfer to microsomes but is inactive with ribonucleoprotein particles unless supplemented with an extract from microsomes. Glutathione appears to be necessary for activity when both the soluble and the microsomal factors are used. The evidence indicates that amino acyl transfer requires at least two protein factors, possibly enzymes; one of these has been obtained from the soluble portion of the cell, and the other has been extracted from the microsomes but is also present in crude soluble fractions. The incorporation of amino acid into RNA associated with the ribosomal particle has also been observed, and the present experiments suggest that the two factors isolated here are involved in this process and in the incorporation of amino acid into protein.

Studies on the incorporation of amino acids into microsomal proteins in cell-free systems have revealed the presence of two distinct enzymatic activities. One of these catalyzes the activation of free amino acids by ATP to amino acyl adenylates and the formation of amino acyl *soluble*-RNA. The isolation and purification of amino acid–specific activating enzymes and the resolution of *soluble*-RNA species indicate the existence of individual enzymes and RNA molecules for different amino acids. The other activity is involved in the transfer of the amino acid moiety of amino acyl *soluble*-RNA to protein (Hoagland *et al.*, 1958; Zamecnik *et al.*, 1958; Grossi and Moldave, 1959, 1960; Takanami and Okamoto, 1960; von der Decken and Hultin, 1960; Nathans, 1960; Nathans and Lipmann, 1960; Bishop and Schweet, 1961; Takanami, 1961; Fessenden and Moldave, 1961); indeed, the incorporation of amino acids from amino acyl *soluble*-RNA into hemoglobin (Hirokawa *et al.*, 1961; v. Ehrenstein and Lipmann, 1961) and albumin (von der Decken and Campbell, 1961) has been reported. A highly purified preparation of this transferring activity that catalyzes amino acid transfer to intact rat liver microsomes in the presence of GTP has been obtained in this laboratory (Grossi and Moldave, 1960; Fessenden and Moldave, 1961). Recent studies (Fessenden and Moldave, 1961) and those reported here with deoxycholate-extracted ribonucleoprotein particles, however, indicate the requirement for an additional protein factor in the transfer of *soluble*-RNA-bound amino acids. This communication describes the effect of soluble preparations and of material extracted from microsomes on this transfer process.

* This work was supported in part by research grants from the American Cancer Society (P-177) and the U. S. Public Health Service (A-1397 and GM-K3-4124).

† U. S. Public Health Service Predoctoral Research Fellow.

EXPERIMENTAL PROCEDURES

A scheme describing the fractionation procedures used to obtain the soluble and microsomal preparations discussed below is presented in Figure 1. The preparation of C^{14}-leucine-labeled *soluble*-RNA from the "*p*H 5 enzyme" fraction and the preparation of washed microsomes from rat liver have been described previously (Grossi and Moldave, 1960). The C^{14}-leucyl *soluble*-RNA used contained approximately 10,000 cpm (3.5×10^{-3} μmoles) of C^{14}-amino acid per mg of RNA. The microsomal preparation did not incorporate amino acid in the absence of a soluble fraction of the cell.

The crude soluble fraction S-II (*p*H 5 supernatant, previously referred to as stage II preparation [Grossi and Moldave, 1960]) and the purified fraction S-V (previously referred to as stage V preparation [Grossi and Moldave, 1960]) prepared from it by fractionation with ammonium sulfate and charcoal, were obtained from the 144,000 × *g* supernatant portion of rat liver homogenates. The *p*H of the solutions was maintained between 6.5 and 7.0 during the salt-precipitation steps, and all dialyses were carried out against several changes of buffered salt-sucrose medium A (0.02 M K_2HPO_4, 0.035 M $KHCO_3$, 0.025 M KCl, 0.004 M $MgCl_2$, 0.35 M sucrose, *p*H 7.0 [Keller and Zamecnik, 1956]) at 4°.

Ribonucleoprotein particles (ribosomes) were prepared from microsomes by extraction with sodium deoxycholate at a final concentration of 0.26% (0.009 M $MgCl_2$) followed by a preliminary centrifugation at 100,000 × *g* for 15 minutes prior to the sedimentation of the ribosomal pellet as described by Kirsch *et al.* (1960). Amino acid was incorporated into the deoxycholate-

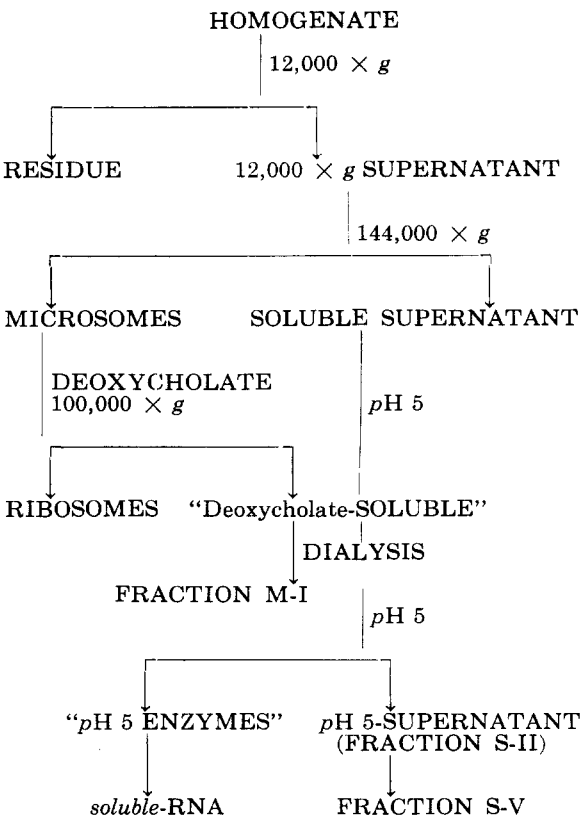

FIG. 1.—Scheme of fractionation procedure.

insoluble ribosomes, while the deoxycholate-soluble fraction was inactive in this respect. The ribonucleoprotein pellets were stored frozen as such or after resuspension in medium A and lyophilization. No loss of activity has been observed over a 6-month period. Occasional ribosomal preparations have been obtained whose activity was approximately 50% of that observed in routine preparations. Although results with low-activity ribosomes are not reported here, their response to various additions to the incubation mixture appeared to be qualitatively similar to that of fully active ribosomes. Fraction M-I refers to the deoxycholate-soluble supernatant prepared from more concentrated (twofold) microsomal suspensions than those used for isolation of ribosomes. This fraction was dialyzed for 3 hours at 4° against several changes of medium A.

Approximately 0.1 mg (1000 cpm) of C^{14}-leucyl *soluble*-RNA, 10 μmoles of ATP, 0.5 μmoles of GTP, ribonucleoprotein particles (containing 2 to 4 mg of protein), and soluble or microsomal preparations as noted were incubated in a volume of 2.0 ml, *p*H 7.0, at 37.5°. The ribosomes were resuspended in medium A immediately before incubation, and the nucleotide preparations contained equimolar concentrations of $MgCl_2$. Unless otherwise specified, incubations were carried out for 30 minutes with 7 mg of fraction S-II protein, 0.007 mg of fraction S-V protein, or 1.2 mg of fraction M-I protein. At the end of the incubation period, the flask contents were chilled, diluted with 2 volumes of cold medium A previously adjusted to *p*H 5, and centrifuged at 144,000 × *g* for 90 minutes to recover the soluble and ribosomal fractions. The *p*H of the diluted suspensions was approximately 6.4, and experiments with unincubated controls or incubations in the absence of transferring preparations indicated that radiocarbon from labeled substrate was not sedimented with the ribosomes under these conditions. In experiments with fraction S-II, approximately 20% of the soluble protein was recovered with the ribosomal pellet when incubation mixtures diluted with *p*H 5 or *p*H 7 medium A were centrifuged at 144,000 × *g*. The sodium chloride (10%, 100°, 30 minutes, *p*H 2–3) extractable nucleic acids and the residual proteins were obtained from perchloric acid–insoluble fractions of the supernatant and ribosomes as described (Moldave, 1960).

RESULTS

Previous studies demonstrated that amino acyl transfer to microsomal proteins required GTP and a soluble portion of the cell (Hoagland *et al.*, 1958; Grossi and Moldave, 1960; Hirokawa *et al.*, 1961). A similar requirement for the transfer to ribosomal proteins has also been observed in this laboratory and others. In the presence of the crude preparation S-II, both the total C^{14} incorporated into ribosomal protein and the rate of incorporation were dependent on the concentration of ribonucleoprotein present in the incubation; two such curves, obtained with ribosomes containing 4 and 6 mg of protein, are shown in Figure 2A. The final specific activity after 60 minutes of incubation, although amino acid uptake was essentially complete at 15 min-

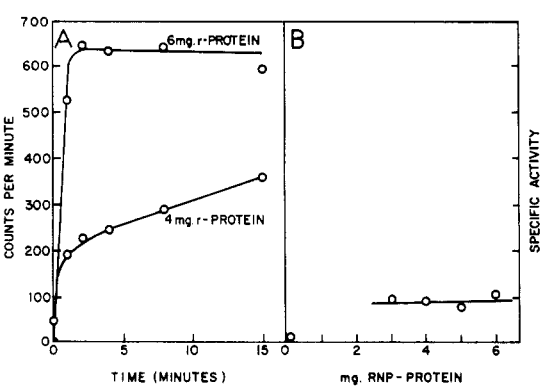

Fig. 2.—Influence of ribonucleoprotein concentration on amino acyl transfer. Incubations carried out in the presence of fraction S-II. *A*, time curves of incorporation with different amounts of ribonucleoprotein. *B*, specific activities (cpm per mg of ribosomal protein) with varying concentrations of ribonucleoprotein; incubation time, 60 minutes.

utes, was approximately 100 cpm per mg ribosomal protein and was independent of the concentration of ribonucleoprotein, as shown in Figure 2B. As in the studies with intact microsomes, incorporation into ribosomes was also proportional to the amount added of soluble preparation S-II.

Although both the crude (S-II) and purified (S-V) soluble preparations catalyzed the transfer reaction in incubations with intact microsomes, in experiments with ribonucleoprotein particles described in Table I the more purified preparation

TABLE I

TRANSFER OF *Soluble*-RNA–AMINO ACID-C^{14} TO INTACT RAT LIVER MICROSOMES AND TO RIBONUCLEOPROTEIN PARTICLES

Additions to Incubation Mixtures[a]	Counts per Minute Incorporated into Protein	
	Microsomes	Ribonucleoprotein
None	0	0
Fraction S-II	218	230
Fraction S-V	163	0

[a] Incubation mixtures contained C^{14}-leucyl *soluble*-RNA (1000 cpm), 7 mg of fraction S-II protein or 0.007 mg of fraction S-V protein, microsomes (4 mg protein) or ribosomes (2 mg protein), and other components as described in the text.

S-V failed to catalyze this process. A preliminary communication (Fessenden and Moldave, 1961) presented evidence that amino acyl transfer was catalyzed by preparation S-V when it was supplemented by the dialyzed deoxycholate-soluble microsomal extract M-I. It was also reported that, although most such preparations were markedly dependent on glutathione, significant incorporation was occasionally observed in the absence of added glutathione (Fessenden and Moldave, 1961). A glutathione requirement in the transfer of amino acid from amino acyl *soluble*-RNA to cytoplasmic particles has been reported previously (Hülsmann and Lipmann, 1960; Nathans and Lipmann, 1960; von der Decken and Hultin, 1960; Bishop and Schweet, 1961). In order to minimize the variations due to the presence or absence of glutathione, the soluble and microsomal extracts and the ribosomes were prepared with solutions made 10^{-3} M with respect to glutathione. Table II summarizes a comparison of preparations thus obtained both with (experiment A) and without (experiment B) glutathione and the effect of glutathione added to incubation mixtures. Whereas fractions S-V and M-I were inactive or only slightly active in both types of preparations when incubated singly or with glutathione, combined fractions S-V and M-I were fully active when glutathione was added to the incubation mixture. Glutathione does not appear to replace either one of the two protein fractions required for the transfer of *soluble*-RNA-bound amino acids. With fractions prepared

TABLE II

THE EFFECT OF SOLUBLE AND MICROSOMAL FRACTIONS ON THE TRANSFER OF AMINO ACID FROM *Soluble*-RNA TO RIBONUCLEOPROTEIN

Additions to Incubation Mixtures[a]	Counts per Minute Incorporated into Protein	
	Experiment A[b]	Experiment B[c]
Fraction S-II	268	202
Fraction S-V	4	56
Fraction M-I	0	20
Glutathione	0	38
Fractions S-V + M-I	155	46
Fractions S-V + M-I + glutathione	273	180
Fractions S-II + M-I	301	
Fraction S-V + glutathione	28	
Fraction M-I + glutathione	0	

[a] Incubation mixtures contained approximately 7 mg of fraction S-II protein, 0.007 mg of fraction S-V protein, 1.2 mg of fraction M-I protein, and 10 μmoles of glutathione where indicated; other incubation components as described in text. [b] Experiment A contained ribosomes, fraction S-II, and fraction M-I prepared with medium A made 10^{-3} M with respect to glutathione. [c] Experiment B contained rat liver preparations obtained with medium A without glutathione.

with solutions containing glutathione, significant activity was also observed when both of these fractions were incubated without added glutathione, probably due to the presence of some glutathione in these preparations.

It should be noted that glutathione had no effect on total amino acyl transfer to intact microsomes catalyzed by preparations S-II or S-V (Grossi and Moldave, 1960), or to purified ribosomes in the presence of preparation S-II described here. Although glutathione has little or no effect on the total incorporation of C^{14} in the presence of preparation S-II, the initial rate of incorporation is markedly stimulated when glutathione is added to incubations consisting of extracts and ribosomes prepared with glutathione-containing solutions. It has also been observed that preparations whose activities were allowed to decay by storing over a period of several months, or some which were initially of low activity, are stimulated by the addition of glutathione.

The observation that the crude soluble preparation S-II is active by itself suggests that both factors S-V and M-I are present in this preparation. The data summarized in Table II show that preparation S-II was also stimulated by the addition of M-I extract. Addition of factor S-V to incubations with fraction S-II had no effect on this transfer process. The stimulatory effect of increasing amounts of fraction M-I on preparation

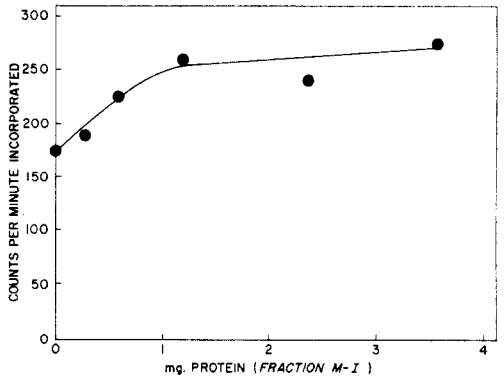

FIG. 3.—Effect of fraction M-I on amino acyl transfer in the presence of fraction S-II.

S-II is shown in Figure 3; these data suggest that the concentration of factor M-I is limiting in the soluble preparation obtained here. A similar conclusion may be drawn from the data presented in Figure 4, which shows the effect of increasing amounts of preparation S-II on amino acyl transfer in the presence and absence of factor M-I. Although a linear response to low concentrations of fraction S-II was not observed in these experiments, the addition of factor M-I markedly stimulated incorporation. It should be mentioned, however, that although fraction M-I has a stimulating effect on fraction S-II, occasionally preparations of fraction S-II have been obtained whose activity is not affected by the addition of fraction M-I; these results probably reflect a certain variability in the concentration of factor M-I in preparation S-II, and are consistent with the observed lability of factor M-I (Fessenden and Moldave, 1961).

Previous studies indicated that labeling of ribosomal RNA was observed in incubations with free amino acid, ATP, and an amino acid–activating enzyme preparation, or with amino acyl *soluble*-RNA and a crude transferring preparation (Moldave, 1960; Fessenden and Moldave, 1962). Amino acid incorporation into microsomal RNA has also been observed with intact ascites cells (Hoagland *et al.*, 1958; Zamecnik, 1958–1959). The incorporation of C^{14}-amino acid into the RNA associated with the ribosomal pellet and the effect of increasing concentrations of fraction S-II, with or without factor M-I, is also shown in Figure 4. The results presented here indicate that, as in the incorporation of amino acid into the protein component, labeling of the RNA moiety is dependent on the concentration of the crude transferring preparation and factor M-I stimulates this process.

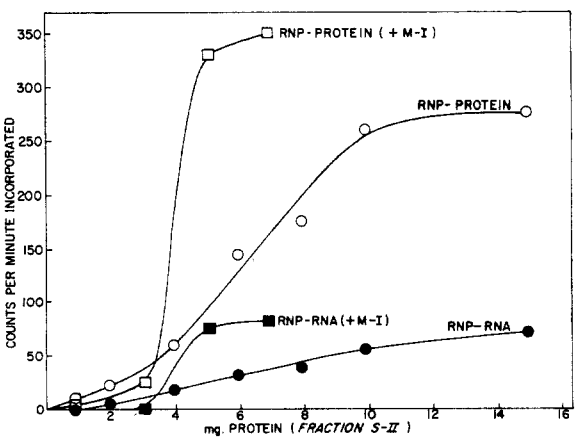

FIG. 4.—Influence of fraction S-II on amino acyl transfer to ribosomal protein (open symbols) and to ribosomal RNA (closed symbols). In each case, the upper curve (+ M-I) represents the effect of fraction M-I on this process.

The effect of varying concentrations of combined fractions S-V and M-I on the transfer of amino acid from amino acyl *soluble*-RNA is shown in Figure 5. The addition of fraction S-V or

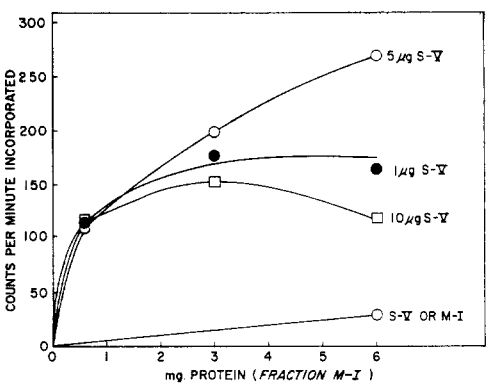

FIG. 5.—Dependence of amino acyl transfer on varying concentrations of combined fractions S-V and M-I. All incubations contained 10 μmoles of glutathione. The lower curve represents fraction S-V or M-I incubated separately; the others were obtained with 1 μg (●—●), 5 μg (○—○), or 10 μg (□—□) of fraction S-V protein as noted.

M-I alone to the incubation did not result in amino acid transfer. In the presence of factor S-V, incorporation is dependent on the concentration of added factor M-I. Similar results have been obtained when fraction M-I is assayed as a function of concentration of factor S-V. However, certain optimal concentrations of these factors are apparent as evidenced by the fact that incorporation in the presence of 10 μg of S-V protein is significantly less than that observed at lower concentrations; it has been observed that an excess of either one of these two factors invariably results in lower incorporation values. Further, studies with intact microsomes and isolated ribonucleoprotein particles have revealed that addition of relatively large amounts of transferring preparations tend to inhibit this process, possibly due to the presence of contaminating inhibitory substances even in the more purified fraction.

Control incubations have revealed occasionally

a preparation of ribosomes capable of accepting amino acids from *soluble*-RNA in the absence of added transferring preparations. Although experiments with such ribosomes are not included here, resuspension of these particles by homogenization in 250 volumes of medium A and resedimentation at 144,000 $\times$ *g* yielded preparations which exhibited an absolute requirement for the transferring factors described above. Thus, as in the studies with intact microsomes (Hoagland *et al.*, 1958; Grossi and Moldave, 1960; Hirokawa *et al.*, 1961), ribosomes have been obtained which are contaminated with soluble components which can be removed by washing procedures. In contrast to the experiments described above, it has been reported that deoxycholate extracts of microsomes catalyze amino acyl transfer to ribosomes in the absence of homogenate soluble preparations (Nathans and Lipmann, 1960). The possibility that these extracts contain both of the activities described here must be considered.

Discussion

The fact that the soluble fraction of the cell is required in the transfer of amino acids from isolated amino acyl *soluble*-RNA to microsomal or ribosomal proteins has now been reported from several laboratories. Partial purification of the transferring activity by chromatography on DEAE-cellulose or calcium phosphate gel columns or by salt-fractionation procedures has been described (Grossi and Moldave, 1960; Takanami and Okamoto, 1960; von der Decken and Hultin, 1960; Bishop and Schweet, 1960; Takanami, 1961; Fessenden and Moldave, 1961). Studies in this laboratory demonstrated that the soluble transferring activity could be greatly purified by means of isoelectric and salt fractionations and treatment with charcoal (Grossi and Moldave, 1960); preparations over 500-fold purified have thus been obtained from the *p*H 5 supernatant fraction (S-II) of rat liver homogenates. Previous studies with intact microsomes indicated that factor S-V, as well as several other enzymes tested (Grossi and Moldave, 1960), was relatively heat-stable (100°, 10 minutes) in the presence of medium A. Dialysis of fraction S-V against 0.02 M phosphate buffer for 3 hours yielded a nondialyzable preparation, containing 50 to 60% of the initial activity, which lost all of its remaining activity when heated for 10 minutes at 60°. Thus, the findings in this laboratory and others (Takanami and Okamoto, 1960; von der Decken and Hultin, 1960; Bishop and Schweet, 1961; Takanami, 1961) that this factor is heat-labile, nondialyzable, and salt-precipitable, as well as its chromatographic behavior, suggest that this activity is enzymic.

Studies with purified transferring factor (Grossi and Moldave, 1960; Takanami and Okamoto, 1960; Bishop and Schweet, 1961; Takanami, 1961) indicated that GTP was the only nucleotide required for amino acyl transfer; this requirement is consistent with the stimulatory effect of GTP, in the presence of ATP, originally observed in the incorporation of free amino acids (Keller and Zamecnik, 1956) and in amino acyl transfer (Hoagland *et al.*, 1958). In the present experiments, ATP has been added routinely as a nucleoside triphosphate–generating system, particularly with crude transferring preparations. However, GTP is indispensible for amino acyl transfer, and in the presence of combined factors S-V and M-I incorporation is obtained with 0.17 μmoles per ml of GTP in the absence of ATP. These results reflect the absence of GTPase activity in these fractions as compared to the crude soluble preparations.

The constancy in the ratios of the specific transferring activities with respect to several amino acids in the process of a 1000-fold purification of the soluble factor has suggested that this enzyme may catalyze the transfer of several or perhaps all of the *soluble*-RNA-bound amino acids to microsomal protein (Grossi and Moldave, 1960). A similar suggestion has been based on the fact that transferring activities toward several amino acids are eluted simultaneously during DEAE-cellulose chromatography (Nathans and Lipmann, 1961).

The transfer of amino acids to the protein component of ribonucleoprotein particles from several sources and the requirement for a soluble transferring factor have been reported. The studies described here with a highly purified preparation of a homogenate supernatant enzyme, however, indicate that an additional factor is required; this has been obtained from microsomes but is also present in crude soluble preparations. Resolution of the two activities described above has been obtained from preparation S-II by salt-fractionation procedures and will be described in a subsequent communication. Studies with the factor designated here as M-I and the one obtained from the soluble fraction which appears to be equivalent to it, but is different from factor S-V, indicate that it is heat-labile (60°, 10 minutes), nondialyzable, and salt-precipitable; these observations suggest that this activity may also be enzymatic. It seems to be rather labile and loses its activity rapidly even when stored frozen.

The incorporation of free and *soluble*-RNA-bound amino acids into ribosomal RNA, and some preliminary studies on the nature of the ribosomal RNA-bound radioactivity, have been reported (Fessenden and Moldave, 1962). Ribosomal RNA labeling has been observed in the incubations described above, and the requirements for this process, to be described in detail subsequently, appear to be quite similar to those for protein labeling. The kinetics of amino acyl transfer to the RNA and protein components of ribonucleoprotein are consistent with a possible role of ribosomal RNA as an intermediary participant in this reaction. The possibility exists that at least two steps are involved in the transfer of the amino acyl group to ribosomal protein, each catalyzed by one of the above enzymes;

however, attempts to dissociate these two labeling processes have not yet been conclusive.

ACKNOWLEDGMENT

We wish to acknowledge the excellent technical assistance of Miss Judith Cairncross.

REFERENCES

Bishop, J. O., and Schweet, R. S. (1961), *Biochim. et Biophys. Acta 49*, 235.
v. Ehrenstein, G., and Lipmann, F. (1961), *Proc. Nat. Acad. Sci. U. S. 47*, 941.
Fessenden, J. M., and Moldave, K. (1961), *Biochem. Biophys. Res. Communs. 6*, 232.
Fessenden, J. M., and Moldave, K. (1962), *Biochim. et Biophys. Acta*, 55, 241.
Grossi, L. G., and Moldave, K. (1959), *Biochim. et Biophys. Acta 35*, 275.
Grossi, L. G., and Moldave, K. (1960), *J. Biol. Chem. 235*, 2370.
Hirokawa, R., Omori, S., Takahashi, T., and Ogata, K. (1961), *Biochim. et Biophys. Acta 49*, 614.
Hoagland, M. B., Stephenson, M. L., Scott, J. F., Hecht, L. I., and Zamecnik, P. C. (1958), *J. Biol. Chem. 231*, 241.
Hülsmann, W. C., and Lipmann, F. (1960), *Biochim. et Biophys. Acta 43*, 123.
Keller, E. B., and Zamecnik, P. C. (1956), *J. Biol. Chem. 221*, 45.
Kirsch, J. F., Siekevitz, P., and Palade, G. E. (1960), *J. Biol. Chem. 235*, 1419.
Moldave, K. (1960), *J. Biol. Chem. 235*, 2365.
Nathans, D. (1960), *Ann. N. Y. Acad. Sci. 88*, 718.
Nathans, D., and Lipmann, F. (1960), *Biochim. et Biophys. Acta 43*, 126.
Takanami, M. (1961), *Biochim. et Biophys. Acta 51*, 85.
Takanami, M., and Okamoto, T. (1960), *Biochim. et Biophys. Acta. 44*, 379.
Von der Decken, A., and Campbell, P. N. (1961), *Biochem. J. 80*, 38 P.
Von der Decken, A., and Hultin, T. (1960), *Biochim. et Biophys. Acta 45*, 139.
Zamecnik, P. C. (1958–1959), *Harvey Lectures 54*, 256.
Zamecnik, P. C., Stephenson, M. L., and Hecht, L. I. (1958), *Proc Nat. Acad. Sci. U. S. 44*, 73.

Cyclo-pseudouridine and the Configuration of Pseudouridine

A. M. MICHELSON* AND WALDO E. COHN

From the Chemist's Laboratory, Arthur Guinness Son and Co., Dublin, Ireland, and Biology Division, Oak Ridge National Laboratory,† Oak Ridge, Tennessee

Received January 29, 1962

Pseudouridine (5-ribosyluracil) C, the naturally occurring isomer, isolated from human urine, has been converted to the O-4:C-5′ anhydro (cyclo) pseudouridine by treating 2′,3′-*O*-isopropylidene-5′-*O*-*p*-toluenesulfonylpseudouridine with sodium *tert*-butoxide. The ether linkage of the product, O-4:C-5′-cyclo-2′,3′-*O*-isopropylidene pseudouridine, is readily hydrolyzed by dilute acid and by alkali to pseudouridine C, which, by virtue of its ability to form the anhydro compound, must have a β configuration and be 5-β-D-ribosyluracil. From this it follows that pseudouridine B is the α anomer.

Pseudouridine, a component of many ribonucleic acids from a variety of sources (Dunn, 1959; Dunn *et al.*, 1960), differs from the other known naturally occurring nucleosides in that the ribosyl linkage is a carbon-carbon rather than a carbon-nitrogen bond (Cohn, 1959). The structure has been established as 5-D-ribosyluracil (Cohn, 1960) and this nucleoside has been synthesized (Shapiro and Chambers, 1961), but the configuration of the glycosyl linkage has not hitherto been demonstrated. Cyclonucleoside formation has been used to establish the β configuration of other natural nucleosides such as the cytidines (Clark *et al.*, 1951; Andersen *et al.*, 1954), thymidine (Michelson and Todd, 1955), and uridine (Brown *et al.*, 1957). The same general method is applicable to pseudouridine, and we report here the accomplishment of such a cyclization and the properties of the product.

Pseudouridine C, the naturally occurring isomer (I in Fig. 1), isolated from human urine (Adler and Gutman, 1959; Adams *et al.*, 1960) by an improved method, was converted into the 2′,3′-*O*-isopropylidene derivative (II) by the method of Yung and Fox (1961). Treatment with *p*-toluenesulfonyl (tosyl) chloride gave the covalent 5′-tosyl derivative (III) which, upon treatment with sodium *tert*-butoxide (Letters and Michelson, 1961), yielded 2′,3′-*O*-isopropylidene O-4:C-5′-cyclopseudouridine (IV). This derivative possesses some of the properties (*e.g.*, acid lability) expected of such a compound (chromatographic and spectral data are given in Tables I and II). The O-4:C-5′ bond is readily hydrolyzed by mild alkali to give 2′,3′-*O*-isopropylidene pseudouridine and by dilute acid to give pseudouridine C.

* Present address: Department of Chemistry, University of Illinois, Urbana.

† Operated by Union Carbide Corporation for the United States Atomic Energy Commission.