Glimcher, M. J., Seyer, J., and Brickley, D. M. (1969), *Biochem. J. 115*, 923.
Kefalides, N. A. (1969), *Arth. Rheum. 12*, 427.
Laurent, T. C., and Pietruszkiewicz, A. (1961), *Biochim. Biophys. Acta 49*, 258.
Mathews, M. B. (1965), *Biochem. J. 96*, 710.
Miller, E. J. (1971), *Biochemistry 10*, 1652.
Miller, E. J., Epstein, Jr., E. H., and Piez, K. A. (1971), *Biophys. Biochem. Res. Commun. 42*, 1024.
Miller, E. J., and Matukas, V. (1969), *Proc. Nat. Acad. Sci. U. S. 64*, 1264.
Miller, E. J., Van Der Korst, J. K. and Sokoloff, Z. (1969), *Arth. Rheum. 21*, 21.
Morgan, P. H., Jacobs, H. G., Segrest, J. P., and Cunningham, L. W. (1970), *J. Biol. Chem. 245*, 5040.
Muir, H. (1958), *Biochem. J. 69*, 195.
Paz, M. A., Blumenfeld, O. D., Rojkind, M., Henson, E., Furfine, C., and Gallop, P. M. (1965), *Arch. Biochem. Biophys. 109*, 458.
Piez, K. A., Eigner, E. and Lewis, M. S. (1963), *Biochemistry 2*, 58.
Roe, J. H. (1955), *J. Biol. Chem. 212*, 335.
Rosenberg, L., Pal, S., Biale, R., and Schubert, M. (1970), *J. Biol. Chem. 245*, 4112.
Sadjera, S., and Hascall, V. (1969), *J. Biol. Chem. 244*, 77.
Schneider, M., and Goldstein, G. (1967), *Proc. Soc. Exp. Biol. Med. 124*, 991.
Spicer, S. S., and Bryant, J. H. (1957), *Amer. J. Pathol. 33*, 1237.
Spiro, R. G. (1969), *J. Biol. Chem. 244*, 602.
Spiro, R. G. (1970), *in* Chemistry and Molecular Biology of the Intercellular Matrix, Vol. 1, Balazs, E. A., Ed., New York, N. Y., Academic Press, p 195.
Stark, M., and Kuhn, K. (1968), *Eur. J. Biochem. 6*, 534.
Stegemann, H. (1967), *Clin. Chem. Acta 18*, 267.
Thomas, L. (1956), *J. Exp. Med. 104*, 245.
Toole, B. P., and Lowther, D. A. (1968), *Arch. Biochem. Biophys. 128*, 567.
Trelstad, R., Kang, A., Igarashi, S., and Gross, J. (1970), *Biochemistry 9*, 4933.
Tsaltas, T. (1958), *J. Exp. Med. 108*, 507.
Warren, L. (1959), *J. Biol. Chem. 234*, 1971.
Zimm, B. H., and Crothers, D. M. (1962), *Proc. Nat. Acad. Sci. U. S. 48*, 905.

Oxidation of the Cysteine-Containing Histone F3. Detection of an Evolutionary Mutation in a Conservative Histone*

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ABSTRACT: All creatures we have examined, including plants, invertebrates, and vertebrates up to and including rodents, contain a single cysteine residue in their F3 histone. Mammals more highly evolved than rodents contain two such cysteine residues. The presence of the additional cysteine affords a more complex oxidation pattern and this has been documented. Thus, in contrast to those F3 molecules which contain a single cysteine residue and have a single dimer oxidation product, the F3 molecules with two cysteine residues can form an intramolecular disulfide bond (in solutions of high dielectric constant or at pH 8.0), a series of dimers with one or two disulfide bonds (in aqueous acetic acid), or a series of higher polymers (at high monomer concentrations). There is no evidence for histone disulfide-bond formation in interphase nuclei.

There are five major groups of histones in the eucaryote nucleus. These can be divided into three types on the basis of their chemistry and their resistance to evolutionary changes in primary structure as measured directly (DeLange *et al.*, 1969) or indirectly using electrophoresis (Panyim *et al.*, 1971), as follows: (1) the arginine-rich histones (F2a1 and F3) in which the primary structure is essentially conserved, (2) the moderately lysine-rich histones (F2b and F2a2) which show a somewhat more frequent change in primary structure during evolution, and (3) the lysine-rich histone (F1), the structure of which is almost species specific.

It is now well recognized that histones from diverse organisms invariably possess a single fraction which contains cysteine (Fambrough and Bonner, 1968; Panyim *et al.*, 1970). Gentle oxidation gives rise to a dimer with a characteristic electrophoretic mobility (Panyim and Chalkley, 1969). Such a dimer is observed as a single electrophoretic band on acrylamide gel electrophoresis. We have observed in this laboratory over the last few years that in contrast to most other species, calf histones give rise to two slower moving electrophoretic bands upon oxidation (Panyim and Chalkley, 1969) as well as developing a species capable of migrating even faster than the parent molecule. Since Bonner had suggested that calf (Fambrough and Bonner, 1968) and also human (Sadgopal and Bonner, 1970) histones contain two cysteine residues, these observations might be explained in terms of two forms of dimeric oxidation products as well as an intramolecular monomer oxidation product.

The following experiments were undertaken to try to establish the molecular nature of the various oxidation prod-

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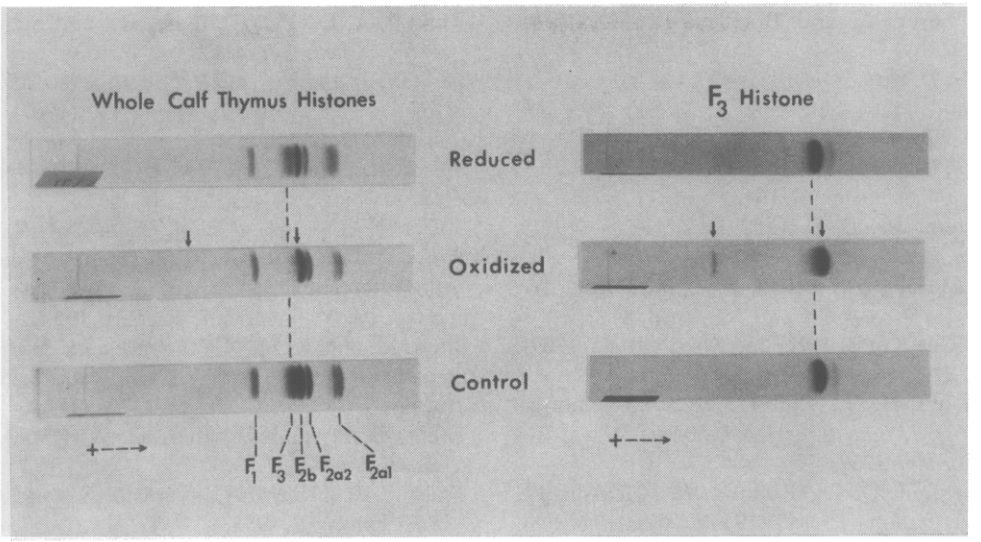

FIGURE 1: Intramolecular oxidation products of histone F3 from calf thymus. Histone F3, either pure (0.3 mg/ml) or as a component of whole histone (1 mg/ml), was dissolved in 6 M urea and oxidized with gentle aeration (see Materials and Methods) at 25° for 12 hr prior to electrophoresis in the standard system (Panyim and Chalkley, 1968). Reduction was with β-mercaptoethanol.

ucts of calf thymus histone and to investigate whether any other mammals contain two cysteine residues in this histone fraction and to assay at which point in evolution the transition from one cysteine to two such residues occurred.

Materials and Methods

Tissues were collected immediately after slaughter and stored at −20°.

Isolation of Histones. Histones were obtained as previously described (Panyim *et al.*, 1971). Histone fraction F3 was obtained by a modification (Panyim *et al.*, 1971) of method 2 of Johns (1964).

Electrophoresis. Polyacrylamide gel electrophoresis followed the procedures previously described (Panyim and Chalkley, 1968). The electrophoretic analysis was run primarily in a urea of concentration 2.5 M. On occasion, gels of different urea molarities were utilized and these were prepared by an appropriate adjustment to the urea concentration in the polymerization solutions.

Oxidation of Histone F3. The histone was dissolved in the solvent of choice (several solvent systems were employed, as described below) and oxidized with gentle shaking so that the surface of the solution was continually disrupted. This procedure is referred to, in the text, as gentle aeration.

Assay for Free Sulfhydryl Groups. Sulfhydryl groups were determined by back-titration using the method of Boyer (1954) or by carboxymethylation with [2-^{14}C]bromoacetic acid (Gurd, 1967). The extent of carboxymethylation was determined by electrophoretic separation of histone fractions followed by sectioning the separated bands and counting in the scintillation counter (Gray and Steffensen, 1968; Bray, 1960).

Results

Intramolecular Oxidized Monomer of F3. If the arginine-rich calf thymus histone (F3) does in fact contain more than one free sulfhydryl group, then the isolation of an intramolecular disulfide-linked molecule would be sufficient proof of this idea. The major factor operating against the formation of such an intramolecularly oxidized monomer of histone F3 would be the high positive charge density of the molecule in aqueous solvents. Since the repulsive effect of the positive charge is reduced in solvents of higher dielectric constant, we have treated samples of both whole histone and pure isolated histone F3 with gentle aeration in the presence of 6 M urea (ε 96) at 25°. In both cases, this gives rise to a new molecular species moving somewhat faster than histone F3, as shown in Figure 1. In the case of whole histone, the new species is not resolved from histone F2b. The experiment using whole histone shows that all other electrophoretic bands are unaffected by this treatment. That the new species is, in fact, histone F3 modified by a disulfide linkage is shown by the observation that treatment with β-mercaptoethanol causes a reversion to histone F3 of normal mobility (Figure 1).

In order to characterize the new species from the oxidation in 6 M urea solution as an intramolecular monomer oxidation product of F3, it was prepared from isolated F3 as described above and its molecular weight compared to that of the parent F3 molecule by exclusion chromatography on Bio-Gel P-100. When the putative oxidized monomer and the parent F3 are jointly applied to a Bio-Gel column, they are eluted at identical positions indicating a close similarity in molecular weight.

The increase in electrophoretic mobility as a result of intramolecular disulfide-bond formation is of the order of 3%. At this time, it is difficult to make a precise assessment of the conformational changes involved in such an increase in mobility, though it seems likely that so small an increase is a reflection of only a small reduction in the effective length of the molecule and as such probably indicates that the sulfhydryl bonds are situated fairly close to one another.

The absence of free SH groups in the faster migrating form of histone F3 (the oxidized monomer) was confirmed by its inability to react with *p*-chloromercuribenzoate and also with bromoacetic acid in a short-term carboxymethylation reaction. The reduced parent F3 molecule, on the other hand, reacts rapidly with *p*-chloromercuribenzoate as shown in Table I. We conclude that there are 2 moles of cysteine/15,000 g of protein. Since the molecular weight of F3 is about 14,500 (Hnilica, 1967; Edwards and Shooter, 1969), it is apparent that there are two cysteine residues per F3 molecule.

TABLE I: Thiol Groups in Calf Histone F3 Molecules.

Sample	Amt (mg)	Mol Wt	Histone (moles)	*p*-CMB[a] (mole equiv)	Calcd SH/Molecule
F3 (reduced)	1.02	15,000	6.8×10^{-8}	1.33×10^{-7}	1.95
F3 (intramolecularly oxidized)	1.19	15,000	7.25×10^{-8}	0.92×10^{-10}	0.0013

[a] *p*-Chloromercuribenzoate.

Intermolecular Oxidation Dimers of F3. Favorable conditions for the formation of oxidation polymers of histone F3 are found by gentle oxidation in solutions of acetic acid. Provided the concentration of histone F3 does not exceed *ca.* 1.0 mg/ml, the parent molecule is almost completely oxidized to two species of dimer, with relatively little higher polymers produced (10–15%). These new species have slightly different electrophoretic mobilities and appear as an electrophoretic doublet with mobility about half that of F3 as seen in Figure 2. That the electrophoretic doublet represents species which are simply oxidation products of histone F3 is seen in the observation that reduction with β-mercaptoethanol converts both bands into the more rapidly migrating parental histone F3 (Figure 2).

The dimer oxidation products of calf histone F3 can be separated from both the parent molecule and from higher polymers by chromatography through Bio-Gel P-100 in 10^{-3} N HCl as shown in Figure 3. Although histone molecular weights are in the region of 10,000–20,000, it is obligatory to use the P-100 gels with a high exclusion volume (for compact proteins) since histones are rather extended particles at the pH and ionic strength of these experiments. In control separations under identical conditions, we applied samples of lysine-rich histone F1 (mol wt 21,000) and also histone F2b (mol wt 13,700). In terms of its point of elution, histone F3 has a molecular weight close to that of histone F2b, which is in agreement with data previously reported (Hnilica, 1967). The oxidized dimer of histone F3 has an apparent molecular weight slightly less than that of the lysine-rich histone, indicating that the molecule is not an end to end dimer but rather that the disulfide bond is nearer the middle.

Although the internally oxidized monomer contains no free sulfhydryl groups as described above, the dimers could possess either (a) two disulfide bonds, or (b) one disulfide bond and

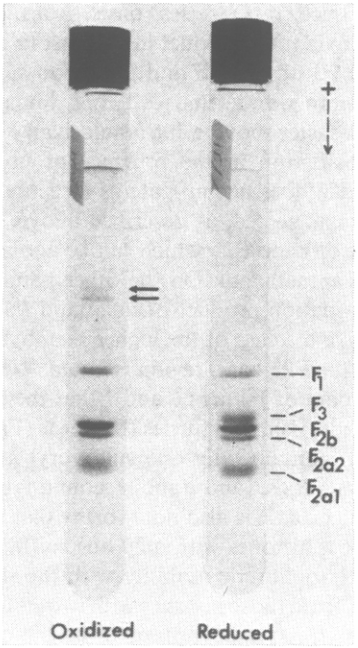

FIGURE 2: Intermolecular oxidation products of histone F3 from calf thymus. A sample of whole histone containing F3 (2 mg/ml) was oxidized for 144 hr at 4° in 0.9 N acetic acid prior to electrophoresis. Reduction was with β-mercaptoethanol.

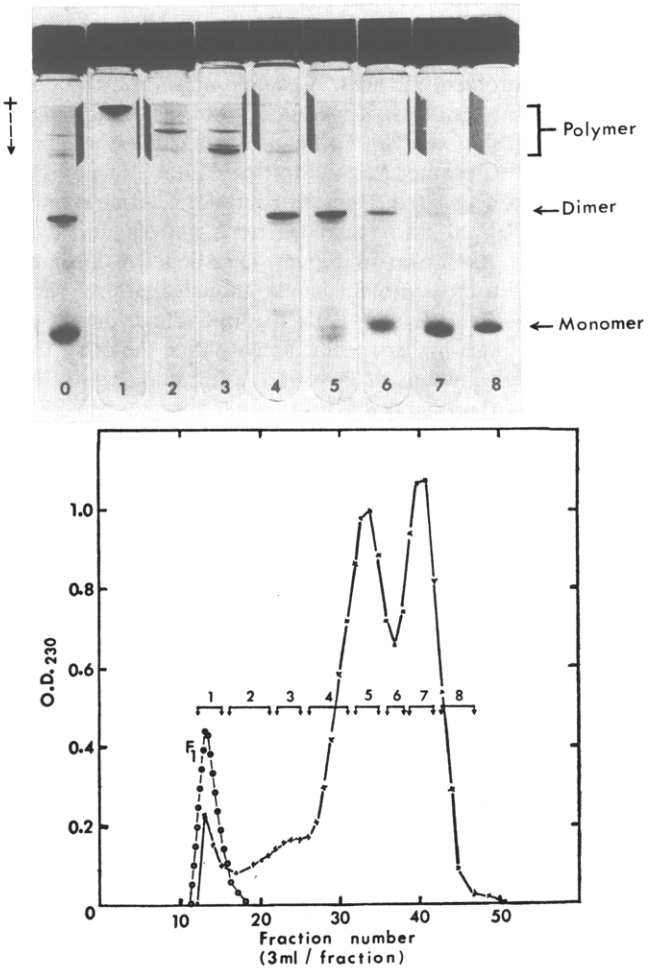

FIGURE 3: Exclusion chromatography of calf thymus histone F3 and its oxidation products. A sample of calf thymus F3 containing about 50% oxidized products was chromatographed on a column of Bio-Gel P-100 in 10^{-3} N HCl (×). In a separate control experiment histone F1 was chromatographed under identical conditions (○). Effluent samples were precipitated in 20% trichloroacetic acid, washed with acetone, and dissolved in 0.9 N acetic acid and applied directly to the electrophoretic system for analysis.

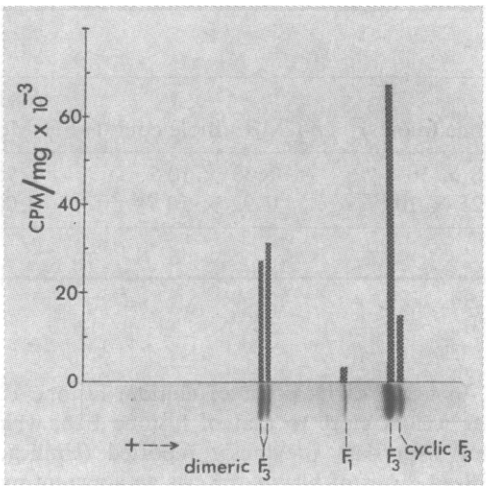

FIGURE 4: Reaction of F3 and its oxidation products with [^{14}C]-bromoacetic acid. A sample of histone F3 containing parent molecule, cyclic intramolecular form, and the dimer forms was incubated with a 5 molar excess of [^{14}C]bromoacetic acid at 25° for 3 hr at pH 7.0. After electrophoresis the bands were sliced and digested in H_2O_2 (Gray and Steffensen, 1968) prior to counting in a scintillation counter.

two free sulfhydryl groups. Accordingly, the oxidized dimers were tested for the presence of free sulfhydryl groups using ^{14}C-labeled bromoacetic acid. A sample of partially oxidized calf thymus F3 histone (with about 50% of histone F3 in the oxidized forms) along with F1 histone as a control was incubated with [^{14}C]bromoacetic acid at pH 7 for 3 hr and then electrophoresed under the normal conditions. After staining, the gel was sliced into equal fractions and examined for radioactivity. As shown in Figure 4, radioactive label was associated with most protein bands, though the level varied considerably. Band 2 and both oxidized dimer bands are labeled to a much higher specific activity than the other fraction; the small amount of label in the intramolecularly oxidized F3 is most probably due to contamination by unoxidized F3 in the gel slice. Under the conditions of the incubation, we would expect sulfhydryl groups to be the most reactive, though methionine residues should show a significant, though low, level of activity toward bromoacetic acid. Thus, we interpret the data as indicating the presence of free sulfhydryl groups in histone F3 and in both of the oxidized dimers. Since each dimer contains four cysteine residues, two of which must be involved in a disulfide linkage, we conclude that each dimer also contains two free sulfhydryl groups.

The picture that emerges then is of two slowly migrating species each consisting of two molecules of histone F3 linked together by a single (but presumably different) disulfide bond and containing in addition two free thiol groups.

Effect of Increasing Urea Concentration on Mobility of Monooxidized Dimer. A sample of calf thymus histone with an appreciable amount of F3 in the form of the oxidized dimers was electrophoresed in gels of different urea concentrations. The results shown in Figure 5A indicate that the faster moving monooxidized dimer is modified by the urea so that it moves more and more slowly until in 6.0 M urea it is migrating almost coincidentally with the slower moving material. Whatever changes are involved can be reversed as is seen by treating histone with 6.0 M urea and then immediately electrophoresing into a gel of lower concentration. The veracity of this last observation, however, is critically dependent upon the time spent in solutions of high urea concentration, as longer exposure to urea gives rise to modifications which are not reversed simply by lowering the urea concentration. The effect of extended urea treatment is to shift the two band dimer system to a single band migrating coincident with the slower band of the normal oxidation product (Figure 5B). Recent studies in this laboratory indicate that after oxidation in urea solutions all sulfhydryl groups in the dimers have been oxidized. We have observed the total absence of thiol groups using either direct titration with [^{14}C]bromoacetic acid or back-titration with *N*-ethylmaleimide.

A Comparison of the Oxidation of Histone F3 from Vertebrates. The rather complex behavior of calf histone F3 upon oxidation (formation of both monomer oxidation product and two dimer oxidation products containing two free thiol groups) is due to the presence of two sulfhydryl groups in the parent molecule. On the other hand (as reported for pea histone (Fambrough and Bonner, 1958)), if a histone contains only one sulfhydryl group it can have only one oxidation product, namely an oxidized dimer containing a single disulfide bond and no free thiol groups.

In principle we could isolate and purify F3 from a range of different creatures and determine the number of cysteine residues by titration against *p*-chloromercuribenzoate. Unfortunately, the isolation of reliably pure F3 is a lengthy and time-consuming procedure. However, by using the methods described above, we have two techniques which permit us to conclude in the presence of all other histone fractions whether a given F3 molecule has one or two cysteine residues per molecule. We can: (1) oxidize slowly in 0.9 N acetic acid and look for the formation of either one or two oxidation dimer bands; or (2) oxidize more rapidly in the presence of 6 M urea and obtain a faster moving intramolecularly oxidized monomer of F3 (coelectrophoresed with F2b) only if two cysteine residues per molecule are present. In addition, this approach would also alert us to the presence of a cysteine residue in another histone fraction if this were ever to occur.

In urea, we have assayed for the internally oxidized monomer, and in acetic acid, we have looked for the number of dimer oxidation products. The data of Figure 6 indicate that the opossum, mice, rats, turtles, birds, frogs, and fish form a single dimer oxidation product in contrast to the two dimers obtained from F3 of the calf and dog upon storage in acetic acid. Furthermore we see that calf, pig, human, and rabbit F3 can form a faster moving intramolecularly oxidized form of F3 upon oxidation in the presence of urea (Figure 7); the new molecule does not migrate as a separate distinguishable band but is detected, as described above, as an increase in the intensity of band F2b which can be abolished by reduction with mercaptoethanol. On the other hand, even in urea solution the oxidation product of duck and fish is the dimer form and (unlike the case of the higher mammals) no increase in intensity of the F2b band region is noted. We deduce therefore from the data of Figures 6 and 7 that the opossum, rats, mice, frogs, birds, fish, and turtles (like peas (Fambrough and Bonner, 1968)) contain only one sulfhydryl group, whereas primates, cows, dogs, and rabbits contain two sulfhydryl groups per molecule. It is also noteworthy that the dimer produced from those histones with only one sulfhydryl group has an identical electrophoretic mobility with the slowest moving dimer obtained from those histones with two sulfhydryl groups.

Since rodents (rats, mice) appear to be the most advanced mammal with a single cysteine residue in the F3 molecule and rabbit the least advanced mammal with two such residues, we decided to obtain additional supporting evidence that this was indeed the point of transition. Pure histone F3

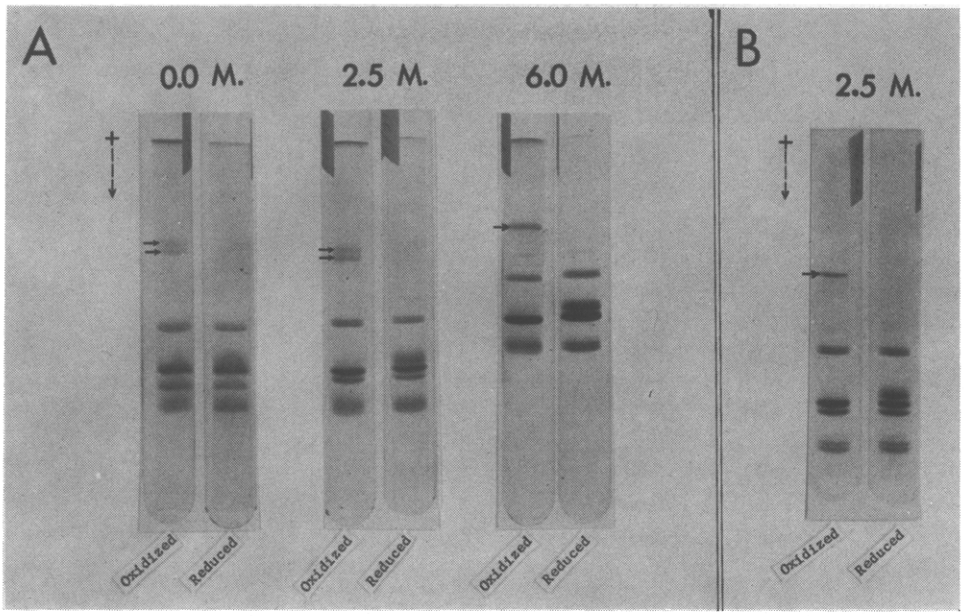

FIGURE 5: (A) Effect of gel urea concentration on the relative mobility of F3 oxidized dimers. Histone was oxidized in 0.9 N acetic acid as described in Figure 2, and applied to gels containing either 0, 2.5, or 6.0 M urea. (B) Effect of extended urea treatment upon dimer oxidation bands of F3 histone. A sample of histone identical with that used in part A (showing two oxidized dimer bands) was dissolved in 6 M urea–0.9 N acetic acid for 24 hr at 4° and then electrophoresed on a gel containing 2.5 M urea—only a single oxidized dimer is seen (arrow).

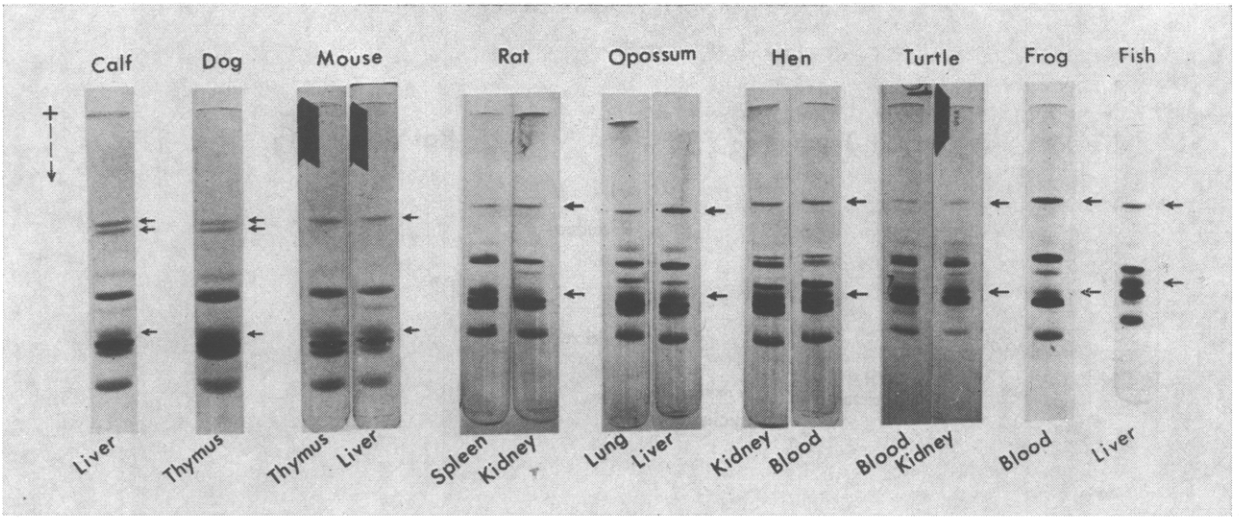

FIGURE 6: An analysis of the oxidation pattern of F3 histone from several vertebrates under conditions favoring dimer formation. The dimer oxidation product was produced as described in the legend to Figure 2. The arrows denote parent F3 histone and also the various dimer products.

was isolated from whole rat and rabbit histone so that we could critically assay for the formation of any internal monomer. Histone was oxidized in urea–acetic acid (the optimum environment for the internal cyclization of calf F3). As shown in Figure 8, rat F3 forms only a single dimer oxidation product and no faster moving cyclic F3 species can be detected. On the other hand, rabbit F3 forms a faster moving cyclic intramolecular oxidation product, providing clear support for the notion that the extra cysteine residue of higher mammals arose after rats had evolved from the main mammalian stem line, but before the divergence of the rabbits.

Discussion

The oxidation behavior of calf thymus F3 histone is more complicated than that observed for many other vertebrate F3 histones. It is characterized by the ability of the calf F3 histones to form either an intramolecularly disulfide-bonded monomer or two forms of dimer molecule which both contain a disulfide bond and two free sulfhydryl groups. Higher molecular weight polymers can also be prepared if the oxidation takes place at high F3 concentrations. That the initial polymer products are in fact dimers is adduced from three lines of evidence. (1) One of the dimer products has an electrophoretic mobility identical with that of the only oxidation product from fish or peas, which have F3 molecules with only a single oxidizable cysteine residue (and are therefore limited to only one oxidation product). (2) Reduction of calf F3 polymers gives rise to the dimer form as an intermediate, whereas the dimer is reduced directly to the mono-

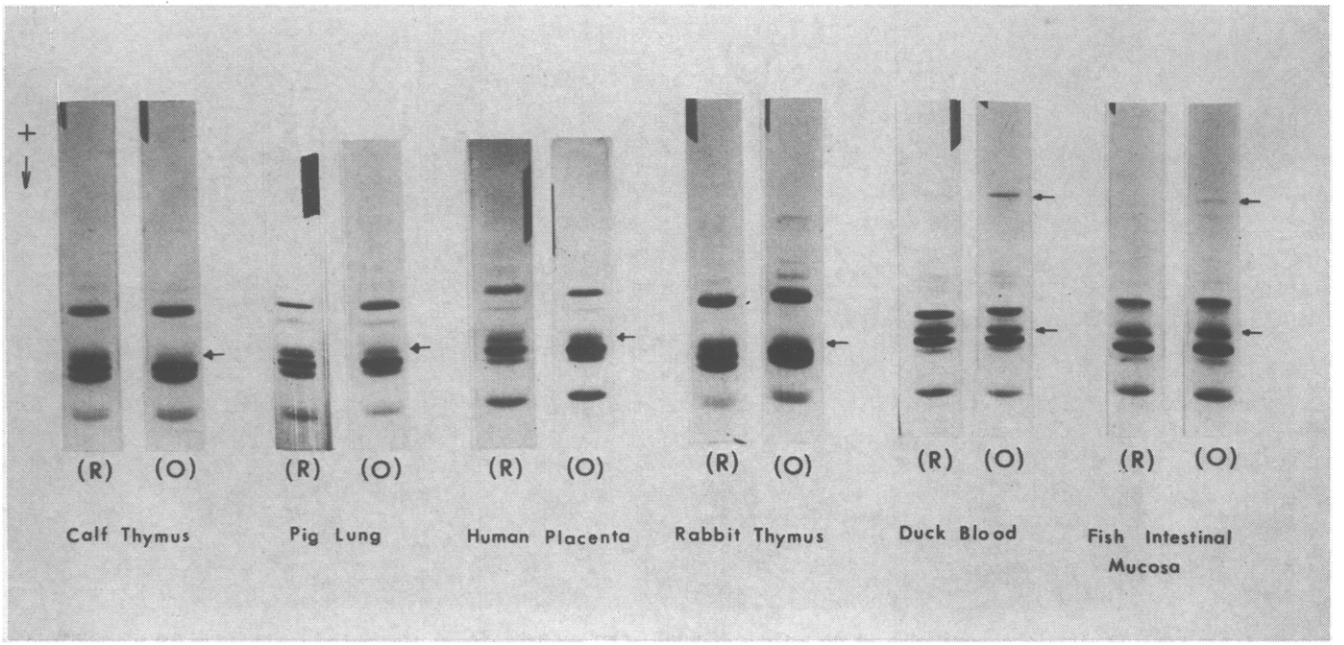

FIGURE 7: An analysis of the oxidation patterns of F3 histone from several verbebrates under conditions favorable for intramolecular oxidation (where possible). Oxidation was as described in the legend to Figure 1. Arrows denote parent F3 and oxidation products (dimers).

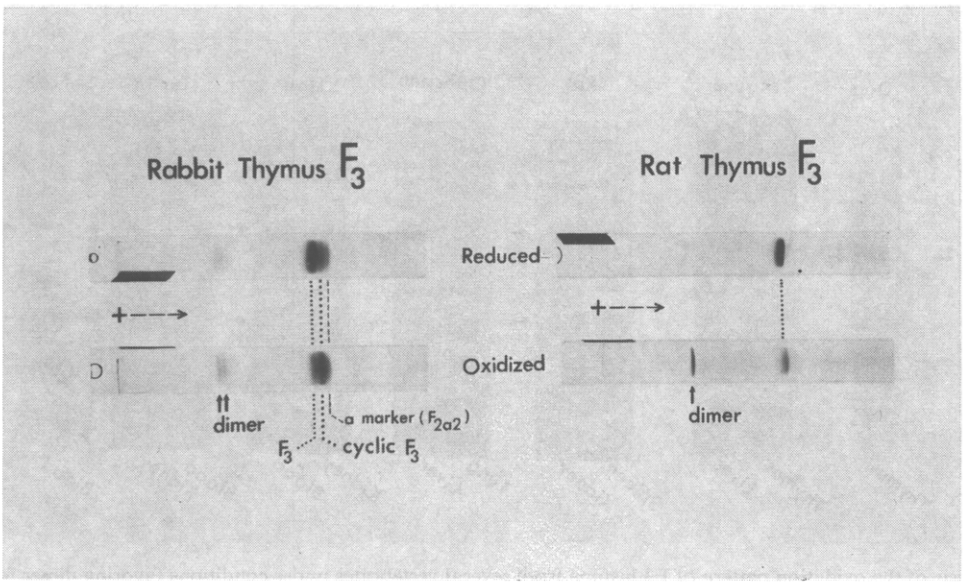

FIGURE 8: Comparison of oxidation behavior of purified F3 from rabbits and rats. Pure F3, isolated from rats and rabbits, was dissolved in 6 M urea–0.9 N acetic acid (conditions for optimal intramolecular disulfide bond formation) and stored at 4° for 4 hr. Electrophoresis of oxidized and control F3 was on our normal gel system (Panyim and Chalkley, 1968).

mer. (3) The dimer oxidation product elutes from Bio-Gel with an apparent molecular weight of about 20,000. The most likely interpretation of this observation is that the disulfide bond joining the monomer F3 molecules is not situated near the end of the molecule.

The formation of the intramolecularly oxidized calf F3 monomer is reflected in a 3% increase in electrophoretic mobility indicating that the effective length of the molecule is not greatly reduced upon internal oxidation. Thus we conclude that the cysteine residues in calf F3 histone are situated fairly close together toward the middle of the molecule.

Essentially all of the F3 molecules isolated by normal procedures are available for oxidation, indicating that the cysteine residues are not bonded through disulfide bonds to other components within the nucleus. The cells we have utilized are almost exclusively in interphase and so we are not able to assay changes in oxidation state in metaphase chromosomes where it has been argued that F3 disulfide bonds are present (Sadgopal and Bonner, 1970).

The large bulk of living creatures examined (including many not reported in this paper) behave as though they contain only a single cysteine residue in the F3 histone fraction. Thus a single oxidized dimer is obtained which migrates with about half the mobility of the parent molecule in the electrophoretic system we employ. This oxidation behavior could be due to three possible causes. (1) The F3 molecule contains two cysteine residues but one is already involved in disulfide bonding, perhaps to a small SH-containing molecule. (2) One

of the cysteine residues is so sterically blocked that it is unable to form a disulfide bond. (3) The F3 molecules exhibiting the simpler oxidation behavior contain only one cysteine residue. The first possibility can be excluded by first reducing the F3 molecule and then assaying for the number of cysteine residues. We have observed no increase in cysteine residues nor any change in the oxidation behavior. The second possibility seems unlikely, especially since isolated F3 histone has little secondary structure at low pH and ionic strength and further since the oxidation behavior characteristic of two cysteine residues is not seen even in the presence of high concentrations of urea. We favor the third explanation and thus conclude that in all creatures with the simpler oxidative behavior (including rodents) there is a single cysteine residue in the F3 histone, whereas more advanced mammalian orders (lagomorphs, carnivores, ungulates, and primates) possess two such residues.

This is a surprising observation. Not only is the mutational introduction of an additional cysteine into a protein a rare event, but also the F3 histone has preserved one of the cysteine residues in all creatures examined so far. Since it has presumably preserved this residue because of the selective advantage it offers, it is curious that the introduction of another cysteine residue relatively close by should not dramatically affect the biological function of the molecule. It will be of interest to inquire whether the two cysteine residues behave in different ways toward sulfhydryl agents when they are part of the nucleoprotein complex. Such experiments are currently under way.

Acknowledgment

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References

Boyer, P. D. (1954), *J. Amer. Chem. Soc. 76*, 4331.
Bray, G. A. (1960), *Anal. Biochem. 1*, 279.
DeLange, R. J., Fambrough, D. M., Smith, E. L., and Bonner, J. (1969), *J. Biol. Chem. 244*, 319.
Edwards, P. A., and Shooter, K. B. (1969), *Biochem. J. 114*, 227.
Fambrough, D. M., and Bonner, J. (1968), *J. Biol. Chem. 243*, 4434.
Gray, R. H., and Steffensen, D. M. (1968), *Anal. Biochem. 24*, 44.
Gurd, F. R. N. (1967), *Methods Enzymol. 11*, 537.
Hnilica, L. S. (1967), *Progr. Nucl. Acid Res. Mol. Biol. 7*, 48.
Johns, E. W. (1964), *Biochem. J. 92*, 55.
Panyim, S., Bilek, D., and Chalkley, R. (1971), *J. Biol. Chem. 246*, 4206.
Panyim, S., and Chalkley, R. (1968), *Arch. Biochem. Biophys. 130*, 337.
Panyim, S., and Chalkley, R. (1969), *Biochemistry 8*, 3972.
Panyim, S., Chalkley, R., Spiker, S., and Oliver, D. (1970), *Biochim. Biophys. Acta 214*, 216.
Sadgopal, A., and Bonner, J. (1970), *Biochim. Biophys. Acta 207*, 227.

Stability of Chromatographic Patterns of Aminoacyl Transfer Ribonucleic Acid from Individual Mouse Plasmacytomas and Variability among Different Immunoglobulin A Producing Plasmacytomas and Normal Organs*

J. Frederic Mushinski

ABSTRACT: The leucyl-, seryl-, and tyrosyl-tRNAs from IgA-producing plasmacytomas and normal tissues were compared using reversed-phase chromatography. The biological stability of these chromatographic patterns was established by comparing the patterns of these aminoacyl-tRNAs from different transplant generations of a single tumor line, which were found to be very similar. Confirmation of the concept that such patterns reflect a stable population of isoaccepting species of tRNA comes from the finding that the tRNAs from two different tumor lines which originated in the same primary mouse have the same patterns for the three aminoacyl-tRNAs examined. Four normal tissues examined in this way showed almost no variation in the chromatographic pattern of Tyr-tRNA, small differences in Leu-tRNA, and somewhat greater differences in Ser-tRNA patterns. Four plasma cell tumors which secrete IgA immunoglobulins with antibody activity showed pattern differences among themselves which were much more striking than the differences among the normal organs. Possible sources of this variable degree of pattern variation are discussed.

A previous report from this laboratory (Mushinski and Potter, 1969) described differences in the relative amounts of each of the five chromatographic peaks of Leu-tRNA when the tRNA from mouse liver and several mouse plasma cell tumors, all of which secreted κ-type immunoglobulin light chains, were compared by reversed-phase column chromatog-

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