MODIFICATION OF POLYURIDYLIC ACID BY BISULFITE: EFFECT ON DOUBLE HELIX FORMATION AND CODING PROPERTIES

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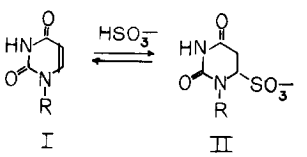
Summary: Polyuridylic acid was allowed to react with 1M sodium bisulfite at 37°C, pH 7, to produce modified polymers in which up to 95% of the uracil residues were converted to 5,6-dihydrouracil-6-sulfonate residues. Partial saturation of polyuridylic acid by bisulfite sharply reduced its ability to form a helical complex with polyadenylic acid, as measured by melting transition and hypochromicity studies. A 31% saturated polyuridylic acid sample did not interact with polyadenylic acid. The ability of polyuridylic acid to code for phenylalanine incorporation in the E. coli cell free protein synthesis system was very markedly diminished by small degrees of bisulfite modification. Reaction to the extent of 2.6% abolished 46% of the phenylalanine coding ability, for example. This indicates that inactivation of messenger RNA may be one pathway by which bisulfite inflicts biological damage.

Sulfur dioxide, and its aqueous form, bisulfite ion, can cause a variety of adverse affects in living organisms, but the biochemical basis of this damage is unknown[1]. The recently described [2,3]uracil saturation reaction (see Scheme 1, I → II) occurs rapidly under physiological conditions and is a likely contributor to bisulfite-induced damage. We wish to report the effect of this reaction upon the ability of polyuridylic acid to form a double helical complex with polyadenylic acid, and to act as a messenger in the E. coli cell free protein-synthesis system.

SCHEME I



I II

Polyuridylic acid (Calbiochem) was allowed to react with 1M sodium bisulfite solution, in 0.1M Tris buffer, at pH 7.02, 37°C. Aliquots were withdrawn periodically and added to excess 0.1M acetate buffer, pH 4, to quench the reaction. The adducts formed (II) were found to be stable for at least one week, at that pH. The ultraviolet absorption of the aliquots fell rapidly as the reaction progressed. They were measured on a Cary Model 15 spectrophotometer, against a suitable blank. As polyuridylic acid shows very slight thermal hyperchromicity and hence base stacking at temperatures above 15°[4], the decrease in absorption was taken to be a direct index of the extent of reaction. The reaction came to equilibrium with 95% of the uracil residues saturated by bisulfite. The loss of absorption was found to follow good first order kinetics, with a rate of 5.27×10^{-4} sec^{-1} (half-life = 21.9 min.). It was possible to prepare polyuridylic acid samples of any desired degree of saturation by interrupting the reaction at the appropriate time, bringing it to pH 4, and removing the excess bisulfite by dialysis at that pH. The modified polymer could be reconverted to polyuridylic acid, with full recovery of the ultraviolet absorbance, by treating it with 0.1M borate buffer at pH 8.9. The reverse reaction also followed first order kinetics, with a rate constant of 2.00×10^{-4} sec^{-1} (half-life = 57.8 min.). Complexes were prepared by mixing polyadenylic acid (P. L. Biochemicals) with bisulfite-modified polyuridylic acid, in 0.01M sodium cacodylate, 0.05M sodium chloride solution, pH 6.38, at 20 or 25° for 15 minutes. The concentration of uracil residues, modified or unmodified, was kept equal to that of the adenine residues. The conditions were chosen to favor double strand (rather than triple strand) formation and to minimize thermal decomposition of the uracil adducts. The melting transitions of the complexes were observed on a Gilford recording spectrophotometer. Representative curves are given in Figure 1. It can be seen that increasing saturation of polyuridylic acid by bisulfite lowers the Tm, broadens the transition, and lessens the hypochromicity. These curves bear a striking resemblance to a set obtained by com-

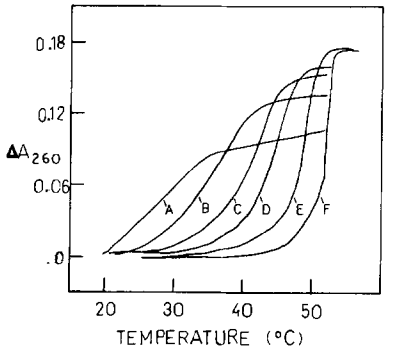


Fig. 1. Melting profiles for complexes of polyadenylic acid and bisulfite-saturated polyuridylic acid. The increase in absorbance (above that of the initial complex) produced on heating is indicated. The per cent modification of the uracil residues is as follows: A, 18.6%; B, 13.9%; c, 7.8%; D, 5.0%; E, 1.9%; F, 0%.

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plexing polyadenylic acid with oligouridylic acids of chain length 8-16 [5]. It appears that the saturation of a uracil residue by bisulfite renders it unable to pair with adenine and is roughly equivalent in effect to causing a chain break. This view is supported by the results of infra-red studies on the melting transition of complexes of Poly rA with polynucleotides containing uracil and dihydrouracil residues[6]. These authors also found a reduction in the binding of the copolymer to poly rA with increasing degrees of saturation. The nature of the complex formed was further studied using continuous variation mixing curves[7]. Mixtures of polyadenylic acid and a partially bisulfite saturated polyuridylic acid were prepared using the above conditions. The molar fraction of polyuridylic acid was allowed to vary from 0 to 100%, each mixture having a constant total nucleotide concentration. The % hypochromicity or per cent decrease in absorbance as compared to that expected for the sum of the constituent polynucleotides at 259 mμ was determined for each sample, one hour after mixing. The results are expressed in Figure 2. The curve for poly rA-poly rU itself showed maximal hypochromicity at concentrations of poly rU above 50%, and was bowed in the range 60-100% U. This behavior is due to the formation of some three-stranded complex in addition to two-stranded complex at compositions above 50% poly rU[8]. As expected, the

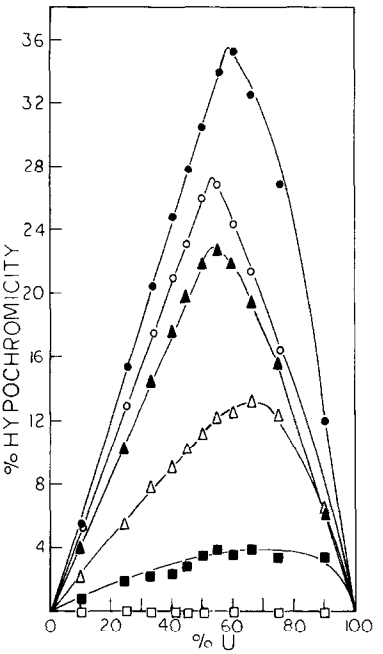

Fig. 2. Mixing curves for polyadenylic acid and polyuridylic acid with the following degrees of bisulfite modification: 0% (●), 7.8% (○), 12.5% (▲), 18.6% (△), 27.5% (■), and 31.5% (□).

absorbance at 280 mμ was found to be constant in the range 0-50% poly rU[9].

It can be seen from Figure 2 that the increasing saturation of poly rU was accompanied by a decrease in the maximum hypochromicity until, at 31% saturation with bisulfite, no further interaction with poly rA was observed. At degrees of bisulfite saturation of polyuridylic acid sufficient to leave only weak binding to polyadenylic acid, the mixture giving maximal hypochromicity contained a large proportion of polyuridylic acid. The polyadenylic acid was presumably selecting only those remaining sequences rich in unmodified uracil, for hybridization.

Phenylalanine incorporation studies were run on bisulfite-modified polyuridylic acid, using the cell-free protein synthesizing system of E. coli. The procedure of Szer[10] was followed. The incorporation of phenylalanine of each modified poly rU was compared to that of a control polyuridylic acid subjected to the same conditions (incubation at pH 7 followed by dialysis at

pH 4) with the replacement of $NaHSO_3$ by NaCl. The control samples incorporated 8700-11,600 cpm (7.84-10.5 mμ moles) above backgrounds, and were unaffected by the above treatment. The modified samples rapidly lost the ability to incorporate phenylalanine into protein with increasing bisulfite content. The results, expressed as % of incorporation activity as compared to the control, were as follows: 2.6% saturation, 54% incorporation; 4.4% saturation, 34% incorporation; 10.5% saturation, 8% incorporation and 16% saturation, 6% incorporation. This striking reduction of the ability of the bisulfite-saturated polymer to promote the incorporation of phenylalanine into protein is in accord with the results obtained in other laboratories using polyuridylic samples modified by reduction[11] or hydroxylamine cleavage[11]. Apparently, the reading mechanism is blocked when it encounters a modified residue [12] and produces very short peptides which do not precipitate with trichloroacetic acid, or no peptides at all[13].

If the sharp loss of activity of polyuridylic acid were entirely due to the saturation reaction, then reversal of the reaction would be expected to restore the activity. An 11.6% saturated sample of polyuridylic acid was allowed to dialyze for 48 hours at pH 8.9, 26°C, against 0.05M borate buffer. Its activity rose to 88% of that of the control (which itself fell in activity to 68% of its previous value.) Thus, the saturation reaction alone appeared to be responsible for the loss of activity. No longer time of reaction was attempted to obtain complete reversal because of the gradual inactivation of the control by the treatment.

In several of the incorporation runs, phenylalanine incorporation was measured in the presence, and absence, of 19 additional unlabeled amino acids. No increase in phenylalanine incorporation was observed in the presence of the additional amino acids. This result indicated that the bisulfite reaction had not conferred any new coding ability to polyuridylic acid. This is parallel to the results obtained with a polymer containing uridylic acid and dihydrouridylic acid[11] but contrasts sharply with the properties of polymers

containing another saturated uracil derivative, uracil photohydrate (6-hydroxy-dihydrouracil). The photohydrate had the ability to be read as cytosine if it occurred in the first or second positions of a codon[15]. It is not clear why this property of uracil photohydrate is not shared by the other dihydro-uracil derivatives.

These results infer that a slight degree of modification of a messenger RNA by bisulfite (perhaps a single uracil saturation) would suffice to block its translation. A similar effect has been observed in the reaction of the potent carcinogen N-acetoxy-2-acetylaminofluorene with synthetic polynucleotide messengers[13]. It is interesting to note also that the introduction of a single uracil photohydrate into the RNA of R_{17} phage is sufficient to inactivate it (although this is not necessarily due to the loss of messenger function by the RNA[16].) At this stage, too little in vivo data is in hand for us to estimate the extent that inactivation of RNA by the adduct (II) contributes to the total biological damage inflicted by bisulfite.

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