Reactive derivative of adenosine-5′-triphosphate formed by irradiation of ATP γ-*p*-azidoanilide

A.G. Badashkeyeva, T.S. Gall, E.V. Efimova, D.G. Knorre, A.V. Lebedev and S.D. Mysina

The Institute of Organic Chemistry, Siberian Division of the USSR Academy of Sciences, Novosibirsk, 630090, USSR

Received 4 March 1983

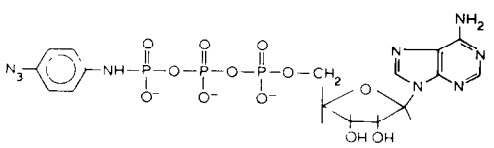
UV and ^{1}H NMR spectra changes demonstrate that *p*-azidoanilides of ATP, ITP, pyrophosphate, after irradiation at 313 nm, transform to similar reactive intermediates. The latter react readily with morpholine, iso-propylamine, *tert*-butylamine, imidazole, mercaptoethanol the reactivity being qualitatively correlated with nucleophilicity. By analogy with the transformation of *p*-azidoanilide, it is concluded that the reactive intermediate is the respective derivative of quinone diimine. Photoaffinity labelling of proteins with *p*-azidoanilide derivatives may proceed as the reaction of the nucleophilic centers of proteins with this quinone diimine derivative, rather than as a direct attack by a nitrene biradical.

Photoaffinity labelling *ATP* *γ-p-Azidoanilide* *Arylazide* *Quinone diimine intermediate*

1. INTRODUCTION

Photoaffinity-labelling is a powerful tool for the investigation of the active sites of biopolymers [1]. The arylazido group is widely used as a reactive moiety of the reagents for photoaffinity-labelling. However, little is known about the chemical structure of the products of interaction of arylazides with proteins under irradiation in aqueous solution. In [2] the first results appeared concerning the intermediates and final products formed in aqueous solution in the photoreaction of arylazides bearing $-CH_2OH$, $-COOCH_3$, $-CONHCH_3$ and $-CHOH-CH(CH_2OH)-NHCOCHCl_2$ radicals in *para*-position to the azido group.

γ-*p*-Azidoanilides of nucleoside-5′-triphosphate were proposed a few years ago as reagents for modification of NTP-dependent proteins [3,4]. Therefore, we have here studied the products of photoconversion of ATP γ-*p*-azidoanilide in aqueous solution. We have found that in the absence of oxygen a highly reactive intermediate accumulates which readily reacts with OH-, NH- and SH-groups typical of the side chains of the amino acid residues of proteins. This product is tentatively identified as the quinone diimine derivative of ATP.



2. MATERIALS AND METHODS

ATP γ-*p*-azidoanilide was obtained as in [5]; ITP γ-*p*-azidoanilide and *p*-azidoanilide pyrophosphate were obtained by similar procedures in 60% and 40% yields, respectively. The products were homogeneous as shown by microcolumn chromatography on a DE-41 Whatman in 7 M urea (pH 7.5) NaCl gradient with negative charge (−3), as well as by thin-layer chromatography (TLC) on Silufol (CSSR) in dioxane:conc. NH_3:H_2O (by vol., 6:1:4) (R_f-values 0.66 for the ITP derivative and 0.78 for the pyrophosphate one). UV-spectra were recorded on spectrophotometer Specord UV VIS (Karl Zeiss, Jena), ^{1}H-NMR spectra were recorded on XL-200 NMR spectrometer (Varian) operating at 200 MHz

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

and 20°C in D_2O solution, chemical shifts are presented relative to tetramethylsilane. Mass spectra were recorded on an AEJ MS 902 spectrometer.

Irradiation of the samples was performed with a mercury lamp at 313 nm, the beam being filtered through BS-4 and UVS-2 filters and saturated $NiCl_2$ solution. The intensity of the resultant light was 1.4×10^{15} quanta.s^{-1}.cm^{-2}. The 10^{-5}–10^{-4} M solutions of the arylazide derivatives were used. The samples were deoxygenated prior to irradiation by 30 min bubbling of argon, purified by alkaline pyrogallol. The addition of nucleophiles to the irradiated arylazido-derivative solution was conducted under permanent argon bubbling. Usually 1 M final conc. of added nucleophile was achieved.

3. RESULTS AND DISCUSSION

When irradiated at 313 nm in the presence of air, ATP γ-*p*-azidoanilide is converted to a mixture of products as revealed by complicated UV-spectral changes without definite isobestic points and with the appearance of an absorption in the visible light. Therefore all experiments were performed with solutions bubbled with argon prior to irradiation.

Fig.1 demonstrates the spectral changes of ATP (a) and ITP (b) γ-*p*-azidoanilides as well as of *p*-azidoanilide of pyrophosphate (c) under irradiation. In all cases the shift to longer wave lengths is observed and typical right-hand shoulders appear. Isobestic points are thus seen to indicate that one main product is accumulated. ^{1}H-NMR spectra presented in fig.2 show that the main changes occur in the aromatic region. These data indicate that the transformation proceeds in the aromatic moiety of the reagent. In the conditions of irradiation used, the conversion is nearly completed in few minutes. The UV-spectra remain unchanged for 15–20 min at 20°C whereupon further changes in the spectra are recorded. This means that the first observed irradiation product (further referred to as derivative A) is rather unstable.

To elucidate whether this unstable derivative may participate in the photoaffinity-labelling we have studied the reaction of derivative A with nucleophiles modelling the typical side chain radicals of amino acids. The addition to the irradiated ATP γ-*p*-azidoanilide of the solution of

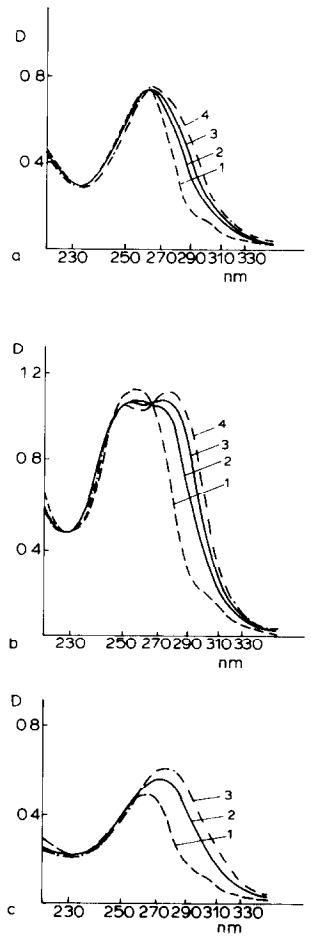


Fig.1. UV-spectra of the irradiated aqueous solutions of *p*-azidoanilides in the course of irradiation: concentration, 2×10^{-4} M; cell width, 0.2 cm; (a) ATP γ-*p*-azidoanilide, irradiation time 0 (1), 60 s (2), 75 s (3) and 90 s (4); (b) ITP γ-*p*-azidoanilide, irradiation time 0 (1), 20 s (2), 40 s (3), 80 s (4); (c) *p*-azidoanilide pyrophosphate, irradiation time 0 (1), 30 s (2), 60 s (3).

morpholine, *tert*-butylamine, *iso*-propylamine, imidazole, β-mercaptoethanol to a final concentration of 1 M results in the UV-spectrum changes thus indicating the interaction of the derivative A with these nucleophiles. With morpholine and β-mercaptoethanol the reaction is accomplished in 1.5 min whereas with imidazole the process continues up to 30 min, with *iso*-propylamine about 40 min and with *tert*-butylamine even 160 min. The common feature of the spectral changes is the disappearance of the right-hand shoulder typical of the derivative A, thus demonstrating that the

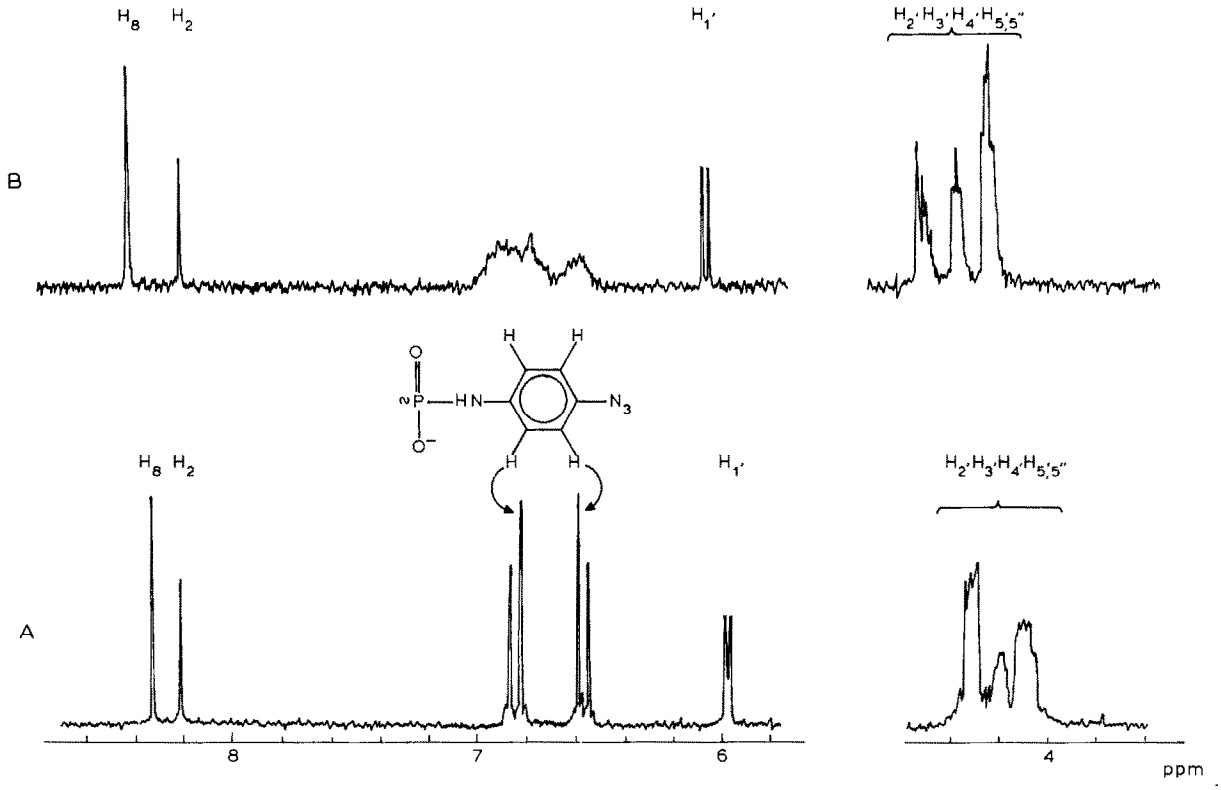


Fig.2. ^{1}H-NMR spectra of 2×10^{-3} M ATP γ-*p*-azidoanilide before (A) and after irradiation (B): 400 accumulations, pulse intervals 4 s, pulse width 4 ms.

structure of the products of interaction of A with the above nucleophiles is closer to that of the starting γ-*p*-azidoanilide. The spectra of the derivative A and the product of the interaction with morpholine are given in fig.3.

The compounds with the same UV-spectra are accumulated when ATP γ-*p*-azidoanilide is irradiated in the presence of these nucleophiles. In fig.4, the spectral changes are represented for the irradiation of ATP γ-*p*-azidoanilide in the presence of *iso*-propylamine. In this case the accumulation of derivative A and its following conversion are easily seen. With morpholine the spectrum of the final product of interaction is already obtained in the first minutes of irradiation.

The whole survey of the data suggests that nitrene biradical which is known to be the primary product of the arylazides irradiation in aqueous solution is easily converted to the relatively long-living intermediate A which reacts readily with nucleophilic compounds.

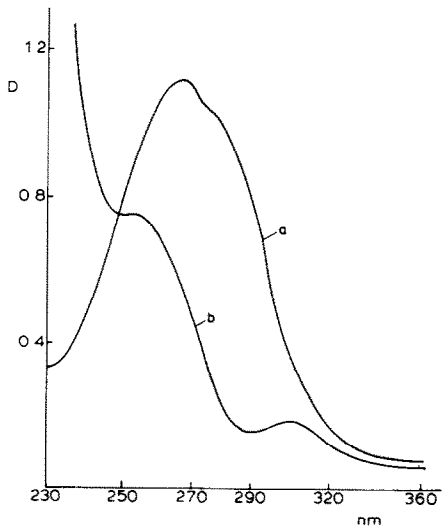


Fig.3. UV-spectrum of the derivative A (a) and of the product of its reaction with 1 M morpholine (b): conc., 2.8×10^{-4} M; l = 0.2 cm.

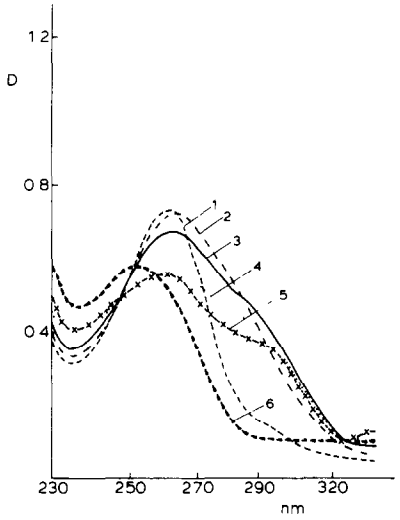
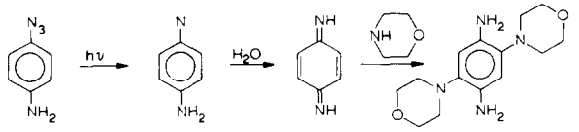
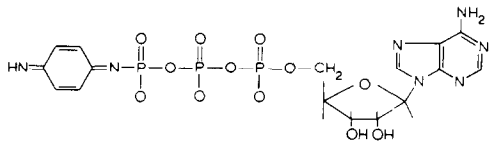


Fig.4. UV-spectra of the irradiated aqueous solution of ATP γ-*p*-azidoanilide in the presence of 1 M *iso*-propylamine before irradiation (1), irradiation time 30 s (2), 90 s (3), 150 s (4), 210 s (5) and additional incubation for 50 min in the dark (6).

In an attempt to elucidate the structure of derivative A we studied similarly the irradiation of *p*-azidoaniline. The UV spectrum of the final product of irradiation is identical with that recorded for quinone diimine described in the literature and easily obtained by oxidation of *p*-phenylene diamine with Ag_2O [6]. In 0.01 M HCl the irradiation product immediately converts to *p*-benzoquinone. In the presence of morpholine, irradiated *p*-azidoaniline converts to *O,O'*-bis-(*N*-morpholinyl)-*p*-phenylenediamine identified by high-resolution mass spectrometry (M_r 278.1705; calc. 278.1742), infrared spectrum (all bands typical of morpholinyl residue, NH-bonds bands at 3350 cm^{-1} and 3340 cm^{-1}), ^{1}H-NMR spectrum (singlet proton signal in aromatic region at δ = 6.2 ppm in $CDCl_3$), positive ninhydrin reaction indicating the presence of amino groups. The transformations observed may be presented by the scheme:



By analogy we can assign to the intermediate A formed by irradiation of ATP γ-*p*-azidoanilide, the structure of quinone diimine derivative:



The shift of the UV-spectrum of ATP γ-*p*-azidoanilide to longer wave lengths under irradiation is in agreement with the formation of the quinoide structure. In favour of the assumption, irradiation of ATP γ-*p*-azidoanilide with subsequent addition of 0.01 M HCl results in the formation of ATP and benzoquinone which may be regarded as a product of acid hydrolysis of benzoquinone diimine.

Irrespective to the real structure of the compound A it seems essential for the affinity-labelling experiments that at least in some cases nitrene biradicals formed under irradiation of the specific complexes of biopolymers with arylazides primarily react with water which may be as accessible to the nitrene attack as the biopolymer groups. The affinity-labelling in this case would proceed with derivatives of type A, not with nitrenes. This means that only nucleophilic groups of the biopolymer would be modified. The widely declared low specificity of photoaffinity-labelling should not be observed in this case.

REFERENCES

[1] Colowik, S.P. and Kaplan, N.O. (1977) in: Photoaffinity-labeling. Methods in Enzymology (Jakoby, W.B. and Wilchek, M. eds) vol.46, pp.69–115, Academic Press, New York.
[2] Nielson, P.E. and Buchardt, O. (1982) Photochem. Photobiol. 35, 317–323.
[3] Grachev, M.A., Knorre, D.G. and Lavrik, O.I. (1981) Soviet Scientific Reviews, section D (Skulachev, V.P. ed) vol.2, pp.107–143, Harwood Academic, London, New York.
[4] Buneva, V.N., Knorre, D.G. and Kudryashova, N.V. (1982) Izv. Sib. Otd. Akad. Nauk SSSR, ser. Khim. Nauk 4, 122–124.
[5] Nevinsky, G.A., Lavrik, O.I., Favorova, O.O. and Kisselev, L.L. (1979) Bioorgan. Khim. 5, 352.
[6] Corbett, J.F. (1969) J. Chem. Soc. B 1, 213–217.