Articles

Antibodies Directed to Drug Epitopes to Investigate the Structure of Drug−Protein Photoadducts. Recognition of a Common Photobound Substructure in Tiaprofenic Acid/Ketoprofen Cross-Photoreactivity

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Drug-induced photoallergy is an immune adverse reaction to the combined effect of drugs and light. From the mechanistic point of view, it first involves covalent binding of drug to protein resulting in the formation of a photoantigen. Hence, determination of the structures of drug−protein photoadducts is of great relevance to understand the molecular basis of photoallergy and cross-immunoreactivity among drugs. Looking for new strategies to investigate the covalent photobinding of drugs to proteins, we generated highly specific antibodies to drug chemical substructures. The availability of such antibodies has allowed us to discriminate between the different modes by which tiaprofenic acid (TPA), suprofen (SUP), and ketoprofen (KTP) photobind to proteins. The finding that the vast majority of the TPA photoadduct can be accounted for by means of antibody *anti*-benzoyl strongly supports the view that the drug binds preferentially via the thiophene ring, leaving the benzene ring more accessible. By contrast, selective recognition of SUP-protein photoadducts by antibody *anti*-thenoyl evidences a preferential coupling via the benzene ring leaving the thiophene moiety more distant from the protein matrix. In the case of KTP, photoadducts are exclusively recognized by antibody *anti*-benzoyl, indicating that the benzene ring is again more accessible. As a result of this research, we have been able to identify a common substructure that is present in TPA−albumin and KTP−albumin photoadducts. This is remarkable since, at a first sight, the greatest structural similarities can be found between TPA and SUP as they share the same benzoylthiophene chromophore. These findings can explain the previously reported observations of cross-reactivity to KTP (or TPA) in patients photosensitized to TPA (or KTP).

Introduction

Drug-induced photoallergy is a particular type of photosensitization and, as such, it involves the development of adverse reactions to the combined effect of drugs and light (*1*, *2*). From the mechanistic point of view, it is generally accepted that photoallergy involves covalent binding of a drug hapten to a protein (haptenization), resulting in the formation of the full photoantigen (*3*). This process is usually initiated by excitation of the drug to a triplet state, which can interact with cell proteins leading to the formation of covalent photoadducts. The resulting photoantigens may trigger a hypersensitivity reaction of the immune system.

Determination of the structures of drug−protein photoadducts is of great relevance to establish the molecular basis of photoallergy and to understand cross-immunoreactivity between two different drugs. Unfortunately, reactions of low molecular weight compounds (i.e., drugs) with large biomolecules (i.e., proteins) are very difficult to investigate (*4*); besides, the degree of functionalization upon photoadduct formation is too low to permit structural studies of modified proteins by conventional methods.

A reliable analysis of photoadducts is difficult to accomplish. Although different methods have been used with variable degree of success, in general, they provide a very limited mechanistic information. Previously used approaches involve either UV−vis spectrophotometric analysis of protein adducts after gel filtration of the irradiated mixtures (*5*) or quantitative measurements of radioactivity irreversibly bound to the protein after irradiation in the presence of labeled drugs (*6*). The former is simple, but not very sensitive and can be

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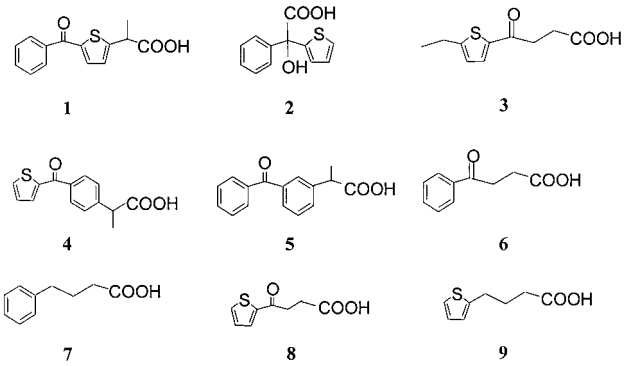


Figure 1. Chemical structures of compounds used to raise/test antibodies. (1) tiaprofenic acid; (2) 2-hydroxy-2-phenyl-2-(2-thienyl) acetic acid; (3) 4-(5-ethyl-2-thienyl)-4-oxobutanoic acid; (4) suprofen; (5) ketoprofen; (6) 3-benzoyl propionic acid; (7) 4-phenylbutyric acid; (8) 3-(2-thenoyl)propionic acid; (9) 4-(2-thienyl)butyric acid.

misleading when the drug chromophore is modified during the process. The latter is clearly more sensitive but is less simple to perform and does not provide full information on the chemical nature of the bound photoantigens.

In recent years, 2-arylpropionic acids have emerged as a remarkable class of nonsteroidal antiinflammatory drugs (NSAIDs) which exhibit diverse biological and pharmacological actions. Unfortunately, some of them (noteworthy tiaprofenic acid, TPA)[1] have shown undesired side effects including photoallergy (*7*, *8*). TPA photobinds efficiently to proteins (*9*, *10*), and this process occurs largely via its decarboxylated photoproduct DTPA (*11*). There is accumulated evidence (*12*) that patients photoallergic to TPA cross-photoreact to ketoprofen (KTP). Reciprocally, KTP-photosensitized patients exhibit cross-photoreactivity to TPA (*7*, *12*−*14*). Surprisingly, this clinical observation is uncommon between TPA and suprofen (SUP), despite the remarkable structural similarity of both drugs (*7*, *15*).

Looking for new strategies to investigate the covalent photobinding of drugs to proteins, we have extended our preliminary work on the use of drug antibodies (*16*, *17*), by generating highly specific antibodies to key chemical substructures of TPA (Figure 1). The primary goal was to gain insight into the chemical nature of the photoadducts formed. Further, as a result of this research, we have been able to recognize a common substructure in TPA−protein and KTP−protein photoadducts that can explain the previously reported observations of cross-reactivity in photosensitized patients.

Experimental Procedures

Materials. Tiaprofenic acid (**1**) was extracted from commercial samples of Surgamic obtained from Rousell Ibérica (Madrid, Spain). Suprofen (**4**) was from Sigma (St. Louis, MO; catalog no. S-9894). Ketoprofen (**5**) was a gift from Laboratorios Menarini (Badalona, Spain). The compounds 2-hydroxy-2-phenyl-2-(2-thienyl) acetic acid (**2**) and 4-(5-ethyl-2-thienyl)-4-oxobutanoic acid (**3**), and were synthesized according to previously established procedures (*18*−*20*). 3-Benzoyl propionic acid (**6**) and 4-phenylbutiric acid (**7**) were from Aldrich (refs B1,1382 and P2,100-5). 3-(2-Thenoyl)propionic acid (**8**) and 4-(2-thienyl)-butyric acid were from Lancaster (Strasbourg, France; refs 3100 and 7563). Decarboxytiaprofenic acid (DTPA) and decarboxysuprofen (DSUP) were prepared by photolysis of TPA and SUP, as previously described (*21*).

Bovine serum albumin fraction V (BSA; catalog no. A-2934), soluble collagen (catalog no. C8897), fibronectin (catalog no. F2006) and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS, catalog no. A-1888) were from Sigma-Aldrich (Madrid, Spain). 1-Ethyl-3-(3-(dimethylamino)-propylcarbodiimide-HCl (EDC) was purchased from Pierce (Rockford, IL; catalog no. 22980). Peroxidase-labeled goat anti-rabbit IgG (catalog no. P0448) was from Dako A/S (Roskilde, Denmark). Hyperfilm (catalog no. RPN3103H) and ECL (catalog no. RPN 2106) were from Amersham Pharmacia Biotech (Madrid, Spain). Bradford protein assay (catalog no. 500-0006) was from Bio-Rad (Richmond, CA). Rabbit serum albumin (RSA) was isolated from rabbit serum by ammonium sulfate precipitation (60% saturation), followed by dialysis and DEAE chromatography (elution in a gradient 0 to 200 mM NaCl, in 5 mM phosphate buffer, pH 7.4, followed by elution with 200 mM NaCl in the same buffer). Fractions eluting at 200 mM NaCl were collected and dialyzed. Fractions were analyzed by SDS−polyacrylamide gel electrophoresis and those containing pure albumin (>98%) were pooled.

Nitrocellulose was from Millipore, Bedford, MA, (catalog no. HAWP304FO). Ninety-six-well microtiter plates for ELISA (Microlon 600; catalog number, 655061), were purchased from Greiner Labortechnik (Frickenhausen, Germany).

Preparation of Drug Antigens. A mixture of 10 mg of rabbit serum albumin, 100 μmol of hapten (compounds **1**−**4**), and EDC (150 μmol) were dissolved in 1 mL of PBS pH 8. The solution was incubated at room temperature for 4 h. Thereafter, the mixture was submitted to gel filtration through a 1 × 15 cm Sephadex G-25 column (20−40 mesh) and eluted with PBS. The eluate was monitored at 280 nm and the first eluting peak containing the protein was collected. To determine the degree of protein functionalization, the UV spectrum was recorded and the absorbance at the wavelength with less interference of albumin was measured (**1**, **3**, 315 nm; **2**, 260 nm **4**, 300 nm). The protein content of the eluate was determined either by densitometry of a coomasie-stained polyacrylamide gel or by the Bradford protein assay and was diluted to about 1 mg/mL. From the difference of the absorbance of adducts measured at the given wavelength and the estimated absorbance of albumin at that wavelength, the amount of covalently bound drug was determined. Typically, the drug/RSA molar ratio of adducts was 10−20.

Production of Drug-Directed Antibodies. Rabbit serum albumin-adducts prepared with compounds **1**−**4** were used to immunize rabbits (3/antigen). The antigens were emulsified with Freund's adjuvant and injected intradermally into rabbits at multiple sites (100 μg of RSA-drug adduct/animal). The procedure was repeated first after 2 weeks and thereafter monthly. After the third immunization, animals were monthly bled (N = 10) and the IgG fraction of serum was isolated by precipitation with ammonium sulfate (50% saturation) followed by dialysis and DEAE chromatography (elution with 5 mM phosphate buffer pH 7.4). The first eluting peak was collected and stored (concentration ca. 1 mg/mL).

Preparation of BSA-Drug Adducts. A mixture of 10 mg of bovine serum albumin, 100 μmol of hapten (compounds **1**−**9**) and EDC (150 μmol) were dissolved in 1 mL of PBS pH 8. The solution was incubated at room temperature for 4 h. Upon gel filtration of the mixture (1 × 15 cm Sephadex G-25 column, 20−40 mesh; eluent PBS), the first eluting peak (monitored at 280 nm) was collected. The degree of protein functionalization was estimated from the UV spectrum by measuring absorbance at the wavelength with less interference of albumin (**1**, **3**, and **8**, 315 nm; **4**, 300 nm; **2**, **6**, **7**, and **9**, 260 nm). The protein content of the sample was determined either by densitometry of a

[1] Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); BSA, bovine serum albumin; DEAE, diethyl-aminoethyl-cellulose; DTPA, decarboxytiaprofenic acid; DSUP, decarboxysuprofen; EDC, 1-ethyl-3-(3-dimethylamino)-propylcarbodiimide; KTP, ketoprofen; PBS, saline phosphate-buffer (20 mM phosphate buffer, pH 7.2, 0.9% NaCl); RSA, rabbit serum albumin; SUP, suprofen; TPA, tiaprofenic acid.

coomasie-stained polyacrylamide gel or by the Bradford protein assay. On the basis of these two parameters, the amount of covalently bound drug was determined as described above. Typically, the drug/BSA molar ratio of adducts achieved was 10−20. Ready to use solutions were prepared by diluting stock solutions with unlabeled BSA, to contain variable amount of drug adduct per milliliter, and ca. 1 mg/mL total BSA.

Assay of Antibody Specificity. Ninety-six-well plates were coated with 100 μL of solutions of increasing concentration of BSA-drug adducts (BSA-**1**, BSA-**2**, BSA-**3**, BSA-**4**), but the same BSA content, dissolved in carbonate buffer (100 mM Na_2CO_3/$NaHCO_3$, 0.02% NaN_3, pH 9.6). Plates were allowed to stand overnight at 4 °C. After thorough washing of plates with plates with PBS/Tween (0.05% Tween-20 in PBS), wells were incubated for 2 h at 37 °C with 100 μL antibodies AbA, AbB, AbC, or AbD, diluted 1:2000 with 0.1% BSA in PBS (equivalent to 50 ng of IgG/well). The use of 0.05% Tween-20 in all solutions prevented unspecific binding of proteins to wells. Upon removal of nonbound antibody by repetitive washing with PBS/Tween (0.05% Tween-20 in PBS), 100 μL of a 1:5000 dilution of peroxidase-labeled goat anti-rabbit IgG in PBS was added (6 ng/well). Plates were then incubated for 1 h at 37 °C. After further washing, 200 μL of a chromogenic solution (0.2 mM ABTS, 2 mM H_2O_2, in 0.15 M citrate buffer, pH 7.2,) was added to each well. Plates were incubated at 37 °C and the absorbance at 405 nm was monitored in a ELISA plate reader (340 ATTC, Labinstruments) until appropriate color development (30−45 min).

The epitope recognition specificity of antibodies "A" and "D" was investigated more accurately using the adducts BSA-**6**, BSA-**7**, BSA-**8**, and BSA-**9** by means of a immunodot assay. Antigens were dotted onto nitrocellulose paper and incubated with antibodies (see below for experimental details).

Irradiation of Proteins with Drugs. Solutions of BSA (0.5 mg/mL in PBS) and drug (0.3 mM) were irradiated in sealed small glass tubes under anaerobic conditions (N_2 atmosphere) for increasing periods of time (0−2 h), using a 125 W medium-pressure mercury lamp. Samples received 1.7 10^{-4} J s^{-1} cm^{-2} during the exposure, as determined by ferrioxalate actinometry (*22*). Similar experiments were carried out using collagen and fibronectin, instead of BSA.

Detection of Drug/Protein Covalent Photoadducts with Antibodies. Samples of the irradiation mixtures were spotted onto nitrocellulose sheets (ca. 5 μg per dot, in 10 μL) and allowed to adsorb for 30 min. The paper was washed several times with PBS/Tween (PBS containing 0.05% Tween-20). Thereafter, the nitrocellulose sheet was saturated for 1 h at room temperature with 5% milk (w/v) in PBS/Tween and incubated for 2 h at 37 °C with 10 mL of a 1/1000 dilution of the antibody in PBS/Tween/milk (equivalent to 10 μg IgG). After repeated washes the blot was incubated with 10 mL of a 1/20 000 dilution of peroxidase labeled goat anti-rabbit (1.5 μg) for 1 h 37 °C, and washed gently with PBS/Tween. Photoadducts were detected using the luminescence detection kit ECL according to the manufacturer's instructions. A high sensitive film (Hyperfilm) was used to detect the immunoblots.

Quantitation of TPA/Protein Photobinding with Antibodies. Aliquots of irradiated TPA/BSA mixtures were dissolved in 100 mM carbonate buffer, pH 9.6, and allowed to adsorb overnight at 4 °C onto ELISA plates (250 ng/well). Plates were washed and incubated for 2 h at 37 °C with a 1:2000 dilution of the drug-directed antibody in a solution containing 1 mg/mL of BSA in PBS. After thorough rinsing with PBS/Tween, plates were incubated for 1 h at 37 °C with a 1:5000 dilution of peroxidase-labeled goat anti-rabbit IgG in PBS. Finally, 200 mL of the chromogenic solution (0.2 mM ABTS 2 mM H_2O_2, in 0.15 M citrate buffer, pH 7.2) was added to each well. Upon incubation at 37 °C, the absorbance at 405 nm was monitored in an ELISA reader after appropriate color development (30−45 min). The extent of photochemical binding was determined by interpolation of absorbance measurements into the appropriate calibration curve.

As ^{3}H-TPA was available, covalent binding was also determined by measuring the radioactivity of ^{3}H-TPA/albumin irradiated samples, as described in detail elsewhere (*16*).

Results

To understand the photochemical processes involved in the formation of TPA photoantigens, a set of specific antibodies directed against substructures of the parent drug was prepared. To this aim, a series of compounds containing drug chemical substructures modeling drug epitopes, and hence potential photoantigens (**1**−**4**, Figure 1), were chemically linked via amide formation to rabbit serum albumin. Upon repeated injection of the adducts into rabbits, immunoglobulins were isolated showing specific recognition to the various drug epitopes.

The characterization of antibodies was done by ELISA, as shown in Figure 2. The results of these experiments allowed the identification of immunoglobulins that specifically recognized different drug epitopes. As summarized in Table 1, antibodies were classified according to their specificity as *anti*-benzoyl (antibody **A**), *anti*-phenyl thienyl carbinol (antibody **B**), *anti*-ethylthenoyl (antibody **C**), and *anti*-thenoyl (antibody **D**).

Qualitative assessment of epitopes in the drug−protein photoadducts was achieved by immune dot assay. As shown in Figure 3A, antibody **A** (*anti*-benzoyl) clearly recognized the BSA irradiated in the presence of TPA or DTPA, but not the photoadducts generated from SUP or DSUP. Conversely, antibody **D** (*anti*-thenoyl) reacted with SUP and DSUP photoadducts, but not with those generated from TPA and DTPA. Interestingly, antibody **A** was also able to recognize KTP photobound to BSA. Antibodies **B** (*anti*-phenyl thienyl carbinol) and **C** (*anti*-ethylthenoyl) were not able to recognize any of the above-mentioned photoadducts. Similar results were obtained when collagen or fibronectin were used instead of BSA.

To confirm that the benzoyl and thenoyl moieties (which include the carbonyl group), rather than the benzyl and thienoyl groups, were actually the substructures recognized by antibodies, an immunodot assay was conducted using antibody "**A**" with antigens BSA-**6** and BSA-**7**, as well as antibody "**D**" with antigens BSA-**8** and BSA-**9** (Figure 3B). The results clearly confirmed the previous assignment.

Immunoquantitation of photoantigens in TPA/BSA irradiation mixtures was carried out by immunoassay using drug epitope-directed antibodies (**A**−**D**). The obtained values were interpolated in the corresponding calibration plots (Figure 4). The results of these experiments were compared with the total amount of photobound TPA as determined by radioactivity measurements (Figure 5). After 2 h of irradiation, ca. 8 pmol of TPA was bound to each mg of protein. Most of it was recognized by antibody **A** (*anti*-benzoyl).

Discussion

Irradiation of TPA in the presence of protein leads to the formation of covalent photoadducts as demonstrated by radioactivity measurements using ^{3}H TPA and recognition by antibodies. The decarboxylated photoproduct DTPA (which shares the benzoylthiophene active chromophore) and Tyr or Trp residues appear to be the key reaction partners involved in adduct formation (*10*). Photobinding must occur via interaction between the

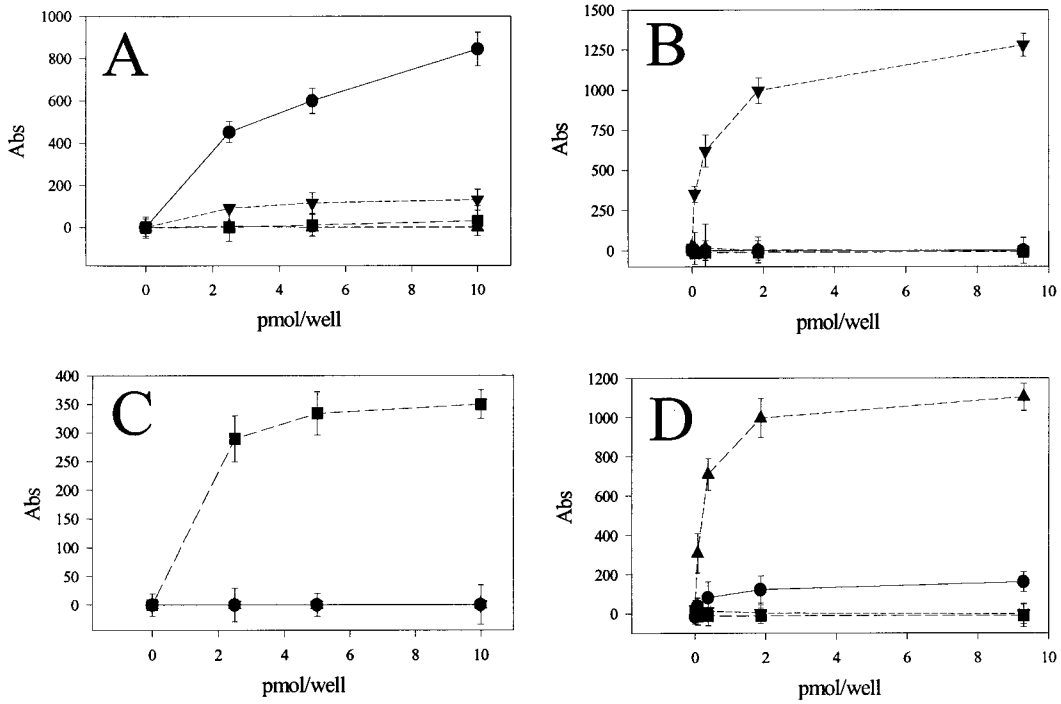
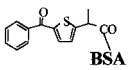


Figure 2. Specificity of drug directed antibodies. Microwell plates were coated with increasing concentrations of **1**-BSA (●), **2**-BSA (▼), **3**-BSA (■), and **4**-BSA (▲) antigens and incubated with drug antibodies. (A) *anti*-benzoyl, (B) *anti*-phenyl thienyl carbinol, (C) *anti*-ethylthenoyl, (D) *anti*-thenoyl. Data represent main values ± SD of three different assays.

Table 1. Classification of Antibodies Regarding Their Specificity and Epitope Recognition

ANTIGEN TESTED	ANTIBODIES A	B	C	D
	****	---	---	---
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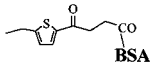
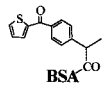
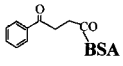
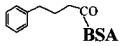
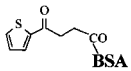
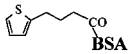
	---	---	****	---
	---	---	---	****
	****	---	---	---
	---	---	---	---
	---	---	---	****
	---	---	---	---
Recognized Epitope	benzoyl	phenyl-thienyl carbinol	ethylthenoyl	thenoyl

DTPA triplet state and the protein. Such interaction leads to formal hydrogen abstraction by the excited chromophore from the phenolic hydroxy group (or the

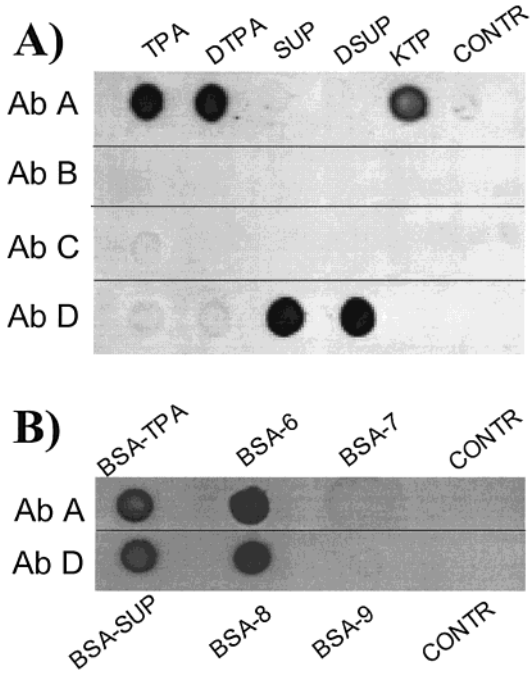


Figure 3. (A) Photobinding of drugs to protein. A solution of BSA (0.5 mg/mL) was irradiated for 2 h in an atmosphere of N_2 in the presence of the drugs (0.3 mM). Samples of the irradiation mixture were applied onto nitrocellulose paper and allowed to adsorb. Parts of the paper were incubated with appropriate antibodies (AbA = *anti*-benzoyl, AbB = *anti*-phenyl thienyl carbinol, AbC = *anti*-ethylthenoyl, AbD = *anti*-thenoyl) and subsequently with peroxidase-labeled *anti*-rabbit IgG antibody. A chemilluminescent substrate (ECL) was used as detection reagent. (B) Substructures recognized by antibodies "**A**" and "**D**". Strips of nitrocellulose paper were coated with 2 pmol of the following adducts: BSA-TPA, BSA-**6**, BSA-**7**, BSA-SUP, BSA-**8**, BSA-**9**. The blots were incubated with antibodies "**A**" (*anti*-benzoyl) or "**D**" (*anti*-thenoyl), and subsequently with peroxidase-labeled *anti*-rabbit IgG antibody. A chemilluminescent substrate (ECL) was used as detection reagent.

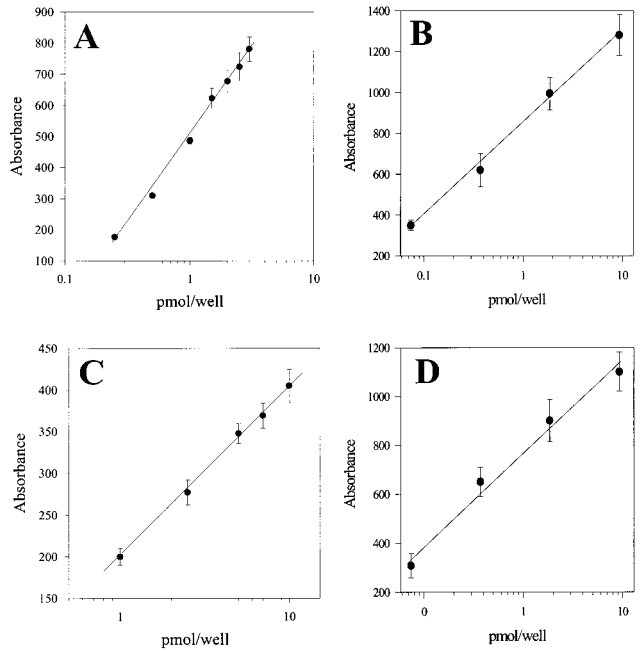


Figure 4. Standard curves for the quantitation of bound photoantigens by ELISA. Different amounts of appropriate drug-BSA adducts were coated on 96-well ELISA plates. Subsequently *anti*-benzoyl, (B) *anti*-phenyl thienyl carbinol, (C) *anti*-ethylthenoyl, (D) *anti*-thenoyl antibodies were added, followed by peroxidase-labeled *anti*-rabbit IgG antibody. ABTS was used as chromogenic substrate, and the absorbance at 405 nm was recorded. Data represent main values ± SD of three different assays.

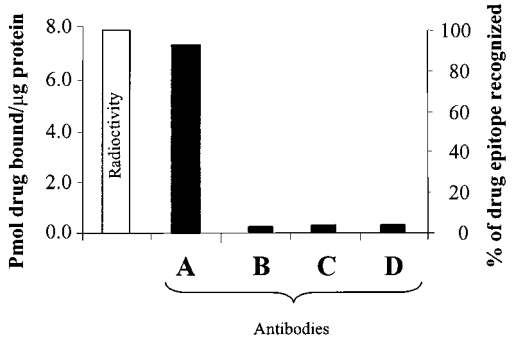


Figure 5. Quantitation of drug/protein photobinding with antibodies. Aliquots of irradiated TPA/BSA mixtures were measured by ELISA using antibodies directed toward drug epitopes. Alternatively, covalent binding was determined by measuring the radioactivity of ^{3}H-TPA/albumin irradiated samples, as described in detail elsewhere (*16*).

indole NH group) of amino acids to give a radical pair. The actual reaction pathway involves an initial electron transfer followed by proton transfer, as expected for a $\pi\pi^*$ triplet (*23*−*26*). Once the radical pair is generated, several possible photoadducts could be formed due to ketyl radical delocalization (Figure 6).

The availability of a set of specific antibodies able to recognize drug substructures has allowed us to discriminate between the different modes of drug photobinding to protein (Figures 3 and 5). The finding that the vast majority of the TPA photoadduct can be accounted for by means of antibody **A** (*anti*-benzoyl) strongly supports the view that the drug binds preferentially via the thiophene ring, leaving the benzene ring more accessible. By contrast, selective recognition of photobound SUP by antibody **D** (*anti*-thenoyl) evidences a preferential coup-

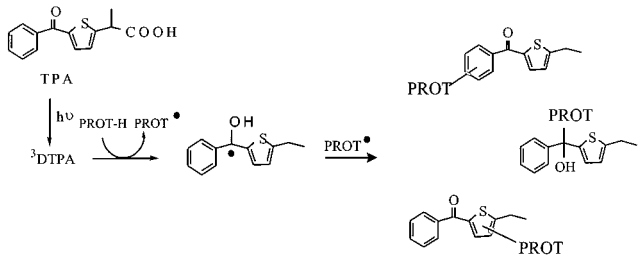


Figure 6. Possible photochemical reactions between TPA and proteins.

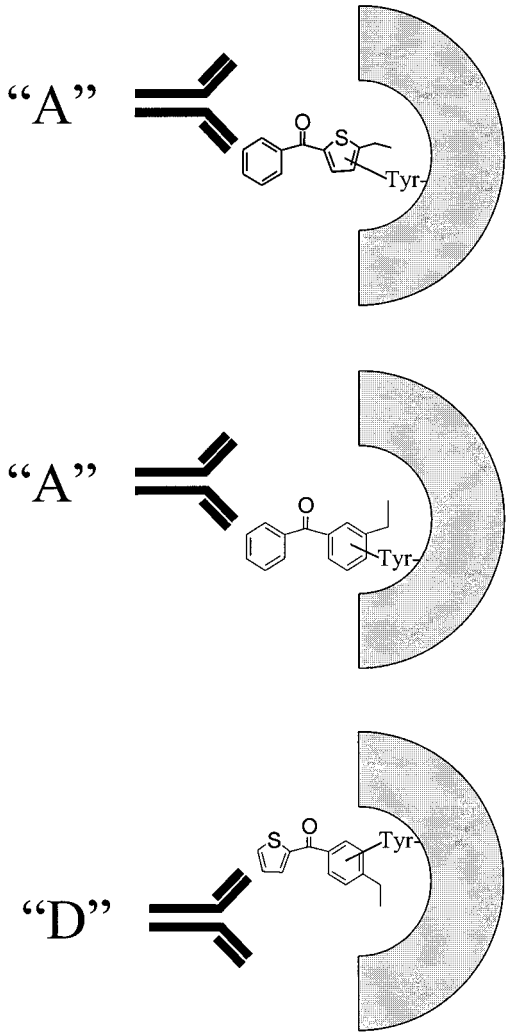


Figure 7. Hypothesized topology and epitope recognition of TPA−, KTP−, and SUP−protein photoadducts.

ling via the benzene ring leaving the thiophene moiety more distant from the protein matrix. In the case of KTP, photoadducts are exclusively recognized by antibody **A**, indicating that the unsubstituted benzene ring is again more accessible. This is outlined in Figure 7.

In summary, specific antibodies directed to drug epitopes have proven to be very elegant tools to investigate the structure of drug−protein photoadducts, which is very difficult to address by other methods. The way in which drugs make covalent bonds to proteins is interesting from the fundamental point of view, but also because of its clinical implications. Thus, as a result of this research, we have been able to identify a common substructure (drug epitope) identified by antibodies in TPA−albumin and KTP−albumin photoadducts but not in SUP−

albumin adducts. This is remarkable since, at a first sight, the greatest structural similarities can be found between TPA and SUP both sharing the same benzoylthiophene chromophore. These findings can explain the reported clinical observations of cross-reactivity to KTP (or TPA) in patients photosensitized to TPA (or KTP) but only rarely to SUP (*7*, *12*−*15*).

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References

(1) Miranda, M. A. (1997) Phototoxicity of Drugs. In *In Vitro Methods in Pharmaceutical Research* (Castell, J. V., and Gómez-Lechón, M. J., Eds.) , Vol. 13, pp 289−315, Academic Press, London.

(2) Spikes, J. D. (1989) Photosensitization. In *The Science of Photobiology* (Smith, K. C., Ed.) 2nd. ed., pp 79−110, Plenum Press, New York.

(3) Lovell, W. W. (1993) A scheme for in vitro screening of substances for photoallergic potential. *Toxicol. In Vitro* **7**, 95−102.

(4) Grossweiner, S. I., and Smith, K. C. (1989) Photochemistry. In *The Science of Photobiology* (Smith, K. C., Ed.) 2nd ed., pp 47−78, Plenum Press, New York.

(5) Barratt, M. D., and Brown, K. R. (1995) Photochemical binding of photoallergens to human serum albumin: a simple in vitro method for screening potential photoallergens. *Toxicol. Lett*. **24**, 1−6.

(6) Miranda, M. A., Castell, J. V., Gomez-Lechon, M. J., Hernández, D., and Martínez, L. A. (1995) Photobinding of drugs to cells as an indicator of potential photoallergy. *Toxicol. In Vitro* **9**, 499−503.

(7) Ophaswongse, S., and Maibach, H. (1993) Topical nonsteroidal antiinflammatory drugs; allergic and photoallergic contact dermatitis and phototoxicity (Review). *Contact Dermatitis* **29**, 57−64.

(8) Hölzle, E., Neumann, N., Hausen, B., Przybilla, B., Schauder, S., Hönisgman, H., Bircher, A., and Plewig, G. (1991) Photopatch testing: the five-year experience of the German, Austrian and Swiss photopatch test group. *J. Am. Acad. Dermatol*. **25**, 59−68.

(9) Miranda, M. A., Castell, J. V., Sarabia, Z., Hernandez, D., Puertes, I., Morera, I. M., and Gómez-Lechón, M. J. (1997) Mechanisms of photosensitization by drugs: involment of tyrosines in the photomodification of proteins mediated by tiaprofenic acid in vitro. *Toxicol. In Vitro* **11**, 653−659.

(10) Miranda, M. A., Castell, J. V., Hernández, D., Gómez-Lechón, M. J., Boscá, F., Morera, I. M., and Sarabia, Z. (1998) Drug-photosensitized protein modifications: identification of the reactive sites and elucidation of the reaction mechanism with tiaprofenic acid/albumin as model system. *Chem. Res. Toxicol*. **11**, 172−177.

(11) De Vries, H., Encinas, S., Miranda, M. A., Castell, J. V., and Beijesbergen and van Henegouwen, G. M. J. (1997) Photodegradation and photobinding of tiaprofenic acid: *In vitro* versus *In vivo*. *Photochem. Photobiol* **66**, 432−435.

(12) Lecoz, C. J., Bottlaender A., Scrivener, J. N., Santinelli, F., Cribier, B. J., Meld, E., and Grosshans, E. M. (1998) Photocontact dermatitis from ketoprofen and tiaprofenic acid. Cross-reactivity study in 12 consecutive patients. *Contact Dermatitis* **38**, 245−252.

(13) Sugiura, M., Hayakawa, R., Kato, Y., Sugiura, K., and Ueda, H. (2000) 4 Cases of photocontactc dermatitis due to ketoprofen. *Contact Dermatitis* **43**, 16−19.

(14) Bagheri, H., Lhiaubet, V., Montastruc, J. L., and Chouini-Lalanne, N. (2000) Photosensitivity to ketoprofen. Mechanisms and pharmacoepidemiologic data. *Drug Safety* **22**, 339−349.

(15) Kurumaji, Y., Ohshiro, Y., Miyamoto C., Keong, C. H., Katoh, T., and Nishioka, K. (1991) Allergic photocontact dermatitis due to suprofen. Photopatch testing and cross-reaction study. *Contact Dermatitis* **25**, 218−223.

(16) Castell, J. V., Hernández, D., Gómez-Lechón, M. J., Lahoz, A., Miranda, M. A., Morera, I. M., Pérez-Prieto, J., and Sarabia, Z. (1998) Photobinding of tiaprofenic acid and suprofen to proteins and cells: a combined study using radiolabeling, antibodies and laser flash photolysis of model bicromophores. *Photochem. Photobiol*. **68**, 660−665.

(17) Miranda, M. A., Sarabia Z., Hernández D., Morera, I. M., Gómez-Lechón, M. J., and Castell, J. V. (1999) An in vitro approach to drug photoallergy: Use of drug-directed antibodies to assess photobinding of non-steroidal antiinflammatories to skin cells. *Toxicol In Vitro* **13**, 701−705.

(18) Blicke, F. F., and Tsao, M. U. (1944) Antispasmodics. VII *J. Am. Chem. Soc*. **66**, 1465−1648.

(19) Papa, D., Schwenk, E., and Hankin, H. (1947) Friedel−Crafts Synthesis of g-Aroyl Aliphatic Acids. *J. Am. Chem. Soc*. **69**, 3018−3022.

(20) Badger, G. M., Rodda, H. J., and Sasse, W. H. F. (1954) Synthetic Applications of Desulphurisation reaction. Part I. The synthesis of fatty acids. *J. Am. Chem. Soc*. **66**, 4162−4168.

(21) Castell J. V., Gómez-Lechón, M. J., Grassa, C., Martínez, L. A., Miranda, M. A., and Tárrega, P. (1994) Photodynamic lipid peroxidation by the photosensitizing nonsteroidal antiinflammatory drugs suprofen and tiaprofenic acid. *Photochem. Photobiol*. **59**, 35−39.

(22) Braun, A. M., Maurette, M. T, and Oliveros, E. (1986) Radiométrie et actinométrie: Actinométrie. *Tecnologie Photochimique*, 1st ed., pp 68−72, Presses Polytechniques Romandes, Switzerland.

(23) Leigh, W. J., Lathioor, E. C., and Pierre, M. J. St. (1996) Photoinduced hydrogen abstraction from phenol by aromatic ketones. A new mechanism for hydrogen abstraction by carbonyl n,π^* and p,π^* triplets. *J. Am. Chem. Soc*. **118**, 12339−12348.

(24) Scaiano, J. C., McGimpsey, W. G., Leigh, W. J., and Jakobs, S. (1987) Kinetic and spectroscopic study of a ketyl-phenoxy biradical produced by remote hydrogen abstraction. *J. Org. Chem*. **52**, 4540−4544.

(25) Arnold, D. R., and Birtwell, R. J. (1973) Photochemical reactivity of some benzoylthiophenes. I. Electronic absorption and emission spectra. *J. Am. Chem. Soc*. **95**, 4599−4606.

(26) Encinas, S., Miranda, M. A., Marconi, G., and Monti, S. (1998) Triplet photoreactivity of the diaryl ketone tiaprofenic acid and its decarboxylated photoproduct. Photobiological implications. *Photochem. Photobiol*. **67**, 420−425.

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