Medical Sciences. In the article “Two types of abnormal genes for plasminogen in families with a predisposition for thrombosis” by Akitada Ichinose, Erik S. Espling, Junki Takamatsu, Hidehiko Saito, Koichi Shinmyozi, Ikuro Maruyama, Torben E. Petersen, and Earl W. Davie, which appeared in number 1, January 1991, of Proc. Natl. Acad. Sci. USA (88, 115–119), the following correction should be noted. On p. 117, Fig. 2A should be as it appears below; Fig. 2B and C were correct as published and are not reproduced.

![Fig. 2](image)

**Fig. 2.** (A) DNA sequence of a portion of exon XV from normal and two abnormal plasminogen genes. (Left) Normal gene. (Center) PLG Nagoya II. (Right) PLG Kagoshima. The arrows indicate the normal guanosines or abnormal adenosines and the asterisk indicates the abnormal adenosine. (B) DNA sequence of a portion of exon XV from a normal individual (Left) and PLG Nagoya I (Right). The arrows indicate the normal guanosines. (C) DNA sequence of a portion of exon X from a normal individual (Left) and PLG Nagoya I (Center and Right). The arrows indicate the normal guanosines or abnormal thymidine and the asterisk indicates the abnormal thymidine.

Biochemistry. In the article “Sorting-out of acceptor–donor relationships in the transglutaminase-catalyzed cross-linking of crystallins by the enzyme-directed labeling of potential sites” by L. Lorand, K. N. Parameswaran, and P. T. Velasco, which appeared in number 1, January 1991, of Proc. Natl. Acad. Sci. USA (88, 82–83), the authors request that the following correction should be noted. The lane designations for EDTA and CaCl₂ in Fig. 1 should be reversed, and the figure legend should read as follows.

**Fig. 1.** Inhibition of crystallin cross-linking in lens homogenate by Dns-Pro-Gly-Gly-Gln-Ile-Val or dansylcadaverine. For the methodology of activating the intrinsic transglutaminase in rabbit lens homogenate, see Materials and Methods. Samples contained no inhibitor (A), 1 μM Dns-Pro-Gly-Gly-Gln-Ile-Val (B), or 2 mM dansylcadaverine (C) and either 2 mM EDTA (lanes 1, 3, and 5) or 10 mM CaCl₂ (lanes 2, 4, and 6). (Upper) Following SDS/PAGE, the gel was photographed under UV light (366 nm; lanes 3–6) and stained with Coomassie brilliant blue R (lanes 1–6). (Lower) Alternatively, the gel was electroblotted and the nitrocellulose sheets were either stained with amido black (lanes 1–6) or immunostained with anti-dansyl IgG (lanes 3–6). The fluorescence on the gels with dansylcadaverine revealed that the band in Upper C, lane 6′, was in fact a doublet. Cross-linked dimeric β crystallins are marked Xβ2.

Biochemistry. In the article “FK506 encodes a nonessential FK 506-binding protein in Saccharomyces cerevisiae and contains regions suggesting homology to the cyclophilins” by Greg Wiedenrecht, Leonardo Brizuela, Keith Elliston, Nolan H. Sigal, and John J. Sicinski, which appeared in number 3, February 1991, of Proc. Natl. Acad. Sci. USA (88, 1029–1033), the authors request that the following correction be noted. Amino acid number 71 in the human FK 506-binding protein sequence in Figs. 3 and 4 should be Q (glutamine) instead of E (glutamic acid).
Sorting-out of acceptor-donor relationships in the transglutaminase-catalyzed cross-linking of crystallins by the enzyme-directed labeling of potential sites

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ABSTRACT The dansyl-conjugated (Dns) peptides Dns-Pro-Gly-Gly-Gln-Gln-Ile-Val and Dns-Ala-Gln-Gln-Ile-Val, patterned on the N-terminal sequence of fibronectin, were synthesized and used for the transglutaminase (protein-glutamine:amine γ-glutamyltransferase, EC 2.3.2.13)-directed selective blocking of lens proteins that otherwise might participate in donating lysyl side chains in forming Nε-(γ-glutamyl)-lysine cross-linked oligomers and polymers. Labeling profiles with these peptides could be readily visualized by fluorescence as well as by immunoblotting with anti-dansyl antibody. The labeling patterns in rabbit lens homogenates were quite different with the dansylated peptides than those obtained with dansylcadaverine. Use of such glutamine-containing dansylated peptides should clearly aid in identifying, isolating, and sequencing potential donor substrates of transglutaminases in many biological systems.

The success of inhibiting cross-linking reactions mediated by transglutaminase (protein-glutamine:amine γ-glutamyltransferase, EC 2.3.2.13) by specifically blocking donor functionalities with glutamine-containing peptide analogues of the N-terminal sequence of fibronectin (1) spurred the current efforts to synthesize similarly acting compounds with readily recognizable reporter groups such as 5-dimethylaminonaphthalene-1-sulfonylethylamine (dansyl) or biotin. First we focused on dansylated derivatives because, in addition to fluorescence detection, methodologies are already in place for immunoblotting and also for the eventual isolation of the tracer-carrying sequences with anti-dansyl antibodies (2, 3).

The present report describes our findings with two dansylated peptides, Dns-Pro-Gly-Gly-Gln-Gln-Ile-Val and Dns-Ala-Gln-Gln-Ile-Val, and demonstrates their usefulness in sorting out the potential acceptor and donor subunits for the cross-linking of crystallins catalyzed by the intrinsic transglutaminase in lens homogenates.

MATERIALS AND METHODS

Dansylated Peptides. Dns-Ala-Gln-Gln-Ile-Val was obtained by treatment of the benzyl ester of Ala-Gln-Gln-Ile-Val (1) with dansyl chloride (Aldrich) in dimethylformamide in the presence of triethylamine, followed by removal of the ester by catalytic hydrogenation. Dns-Pro-Gly-Gly-Gln-Gln-Ile-Val was synthesized by coupling of the tripeptide Dns-Pro-Gly-Gly to the benzyl ester of Gln-Gln-Ile-Val (1), followed by hydrogenation.

Inhibition of Crystallin Cross-Linking in Lens Homogenate. Frozen lenses from young rabbits (Pel-Freez Biologicals) were thawed and decapsulated, and the cortex was separated from the nucleus. The cortical portions from three lenses were homogenized in 2 ml of 50 mM Tris-HCl, pH 7.5/100 mM NaCl by hand in a Potter-Elvehjem tissue grinder. Incubations were carried out at 37°C in a total volume of 100 μl containing homogenate (approximately 50 mg of protein per ml), 20% (vol/vol) glycerol, 2 mM leupeptin (obtained through the U.S.-Japan Cooperative Cancer Research Program), 2 mM of one of the dansylated peptides or dansylcadaverine (cadaverine is 1,5-diaminopentane), and either 8 mM CaCl₂ or 2 mM EDTA. Mixtures were incubated for 15 min at 37°C prior to the addition of one of the latter two components. The reactions were then allowed to proceed at the same temperature for a period of 90 min, when they were stopped by the addition of 20 μl of 100 mM EDTA. The samples were centrifuged (15,600 × g for 5 min), and 10 μl of the supernatants were incubated with 100 μl of 50 mM Tris-HCl, pH 7.1/9 M urea/2% sodium dodecyl sulfate (SDS)/40 mM dithiothreitol for 60 min. Approximately 130 μg of proteins were analyzed by SDS/PAGE using the discontinuous buffer system of Laemmli (4) on 1.5-mm-thick gels in a protein slab gel apparatus (Bio-Rad). A stacking gel of 3% acrylamide, a resolving gel of 12% acrylamide, and a running buffer of 25 mM Tris/192 mM glycine/0.1% SDS, pH 8.6, were used. The gel was stained with Coomassie brilliant blue R and was calibrated with molecular weight standards (Bio-Rad) of phosphorylase b (Mr 97,400), bovine serum albumin (Mr 66,200), ovalbumin (Mr 45,000), carbonic anhydrase (Mr 31,000), soybean trypsin inhibitor (Mr 21,500), and lysozyme (Mr 14,400). Electroblotting (75 μg per lane) to nitrocellulose (0.2 μm; Schleicher and Schuell) was carried out with a LKB model 2005 electroblotting unit for 2 hr at 4°C by the method of Towbin et al. (5) in 25 mM Tris/192 mM glycine, pH 8.3/20% (vol/vol) methanol. For purposes of immunostaining, a rabbit IgG to dansylated bovine gamma globulins was diluted 1:5000 into 10 mM sodium phosphate, pH 7.1/0.9% NaCl/0.05% Tween 20 and a Vectastain ABC kit (Vector Laboratories) was used for the peroxidase-based staining (6).

RESULTS AND DISCUSSION

The cross-linking reaction in rabbit lens homogenate, as catalyzed by the intrinsic transglutaminase upon addition of Ca²⁺, is of particular interest because—among all the cytoplasmic proteins present—only a small population of β crystallin subunits are involved. None of the α or γ crystallins seems to participate directly in forming Nε-(γ-glutamyl)-lysine-linked structures (7, 8). Kinetically, the first cross-linked products are the dimeric β crystallins and these were followed by formation of higher ordered oligomeric and polymeric structures. Protein profiles on SDS/PAGE suggest that generation of dimeric β crystallins is not a homologous fusion of two identical β crystallin subunits.

Abbreviation: Dns, dansyl conjugated to peptides.

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Previously with the use of dansylcadaverine, while blocking the formation of dimeric β crystallins, we were able to label only the β crystallin subunits that acted as the potential amine acceptor partners in the transglutaminase-mediated reaction. These effects of dansylcadaverine are also demonstrated in Fig. 1C. The recent observation that certain endo-glutamine-containing peptides, patterned on the N-terminal sequence of fibronectin, could inhibit cross-linking in the lens system while selectively modifying some crystallins (1) offered the opportunity to identify the potential amino group-donating protein subunits.

Given the analytical advantages afforded by a dansyl probe, we prepared two peptides carrying the fluorescent and haptenic moiety: Dns-Pro-Gly-Gly-Gln-Ile-Val and Dns-Ala-Gln-Gln-Ile-Val. Fig. 1B presents our findings with the former compound; results with the other derivative were quite similar, though the impression was gained that inhibition of cross-linking was somewhat better with Dns-Pro-Gly-Gly-Ile-Val. Formation of dimeric β crystallins could, in fact, be inhibited by as little as 0.5 mM of this compound.

The most important observation was that the transglutaminase-mediated labeling pattern of proteins with dansylcadaverine was quite different from that obtained with the glutamine-containing dansylated peptides. This is proved by fluorescence detection (Fig. 1 Upper) as well as anti-dansyl antibody-dependent immunostaining (Fig. 1 Lower). The obvious conclusion is that dansylcadaverine visualizes the potential acceptor sites, whereas the dansylated peptides specifically mark the donor proteins that could conceivably contribute the lysyl side chains to the N\(^\text{+}\)(γ-glutamyl)lysine bridges in heterologous cross-linking.

As previously noted (1), peptide-decorated crystallins undergo an upward shift in SDS/PAGE to positions of apparent higher molecular weight values depending on the number of transglutaminase-reactive donor groups actually blocked, and this was also seen with the dansylated compounds. Hence, some of the multiplets visualized in lanes 4' of Fig. 1B could represent different degrees of modifications of the same protein subunit. Nevertheless, a comparison of the labeling patterns with the glutamine-containing dansylated peptides and dansylcadaverine (in Fig. 1, compare lanes 4' in B with lanes 6' in C) suggests that the acceptor subunits, marked with the latter probe, rather than donors may be the limiting component for generating dimeric β crystallins.

Use of such glutamine-containing dansylated peptides in an analogous manner will undoubtedly also aid in identifying, isolating, and sequencing potential donor substrates of transglutaminases in other biological systems.

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