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A 43 kDa form of the GTP-binding protein G_{i3} in human erythrocytes

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Purified preparations of human erythrocyte G-proteins contain a 43 kDa pertussis toxin substrate which appears to be the α-subunit of a heterotrimeric GTP-binding protein. The 43 kDa protein is recognized by antisera that are sequence-specific for peptides encoding a sequence common to all 39–53 kDa G-protein α-subunits. $G\alpha_o$-specific antiserum did not recognize 43 or 40–41 kDa α-subunits. AS/6, which recognizes the α_i proteins, recognized 43 kDa as well as 40–41 kDa proteins. Of the three antisera specific for individual members of the α_i family, only the G_{i3}-specific antiserum recognized the 43 kDa erythrocyte G-protein. However, 40–41 kDa forms of all three α_is are present. These observations indicate that human erythrocytes contain a novel 43 kDa form of G_{i3}.

Guanine nucleotide binding protein, 43 kDa; Pertussis toxin; Human erythrocyte

1. INTRODUCTION

Heterotrimeric GTP-binding proteins (G-proteins) serve to couple hormones and neurotransmitter receptors to a variety of effectors. Several families of G-proteins have been identified by cDNA cloning [1]. In most tissues, the G-proteins that are pertussis toxin sensitive fall into two distinct categories, G_i or G_o. Three distinct types of G_i have been identified and shown to be separate gene products [2,3]. Similarly, it appears that there will be multiple forms of G_o [4,5]. During the course of our purification of human erythrocyte G-proteins we had identified a 43 kDa pertussis toxin substrate [6]. We have now characterized the 43 kDa protein and found that it is a high molecular weight form of G_{i3}.

2. MATERIALS AND METHODS

The sources of most materials have been previously described [6–8]. Antisera A-569, J-881, U-46, H-660, I-355, J-883, and K-887 were the kind gift of Drs S. Mumby and A.G. Gilman. Antiserum AS/6 was the kind gift of Dr A. Spiegel. The specificities of all these antisera have been verified using recombinant α-subunit fusion proteins expressed in *E. coli*. Protocols for such verification have been described in detail elsewhere [7,8].

2.1. *Purification of human erythrocyte G-proteins*

Human erythrocyte G-proteins were purified through the second DEAE-Sephacel step [9]. At this stage we have a mixture of G-proteins that is essentially free of contaminants. This mixture of G-proteins was used for immunoblotting.

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2.2. *Immunoblotting studies*

Details of the procedures used for immunoblotting analysis in our laboratory have been published elsewhere [6–8].

2.3. *Replication of results*

All experiments have been repeated twice using two different batches of G-protein.

3. RESULTS

Previous studies had shown that the human erythrocyte contained a 43 kDa pertussis toxin substrate [7], but the identity of the 43 kDa protein was not determined. In order to ascertain that the 43 kDa protein was an α-subunit we blotted it with two antisera, A-569 and J-881, made to the common identity box region within the guanine nucleotide binding pocket. This region is conserved for all α-subunits from yeast to humans [1]. Both α-subunit specific antisera recognized the 43 kDa protein as well as proteins in the 40–41 kDa range (fig.1).

We tested this for several different preparations of G-proteins and found that they all contained 43 kDa protein(s) that was immunoreactive with the α-common antibody. We next determined whether the human erythrocyte G-proteins were recognized by α_o- or α_i-specific antisera. U-46, an α_o-specific antiserum [10], did not recognize any of the human erythrocyte G-proteins. In contrast, AS/6, an antiserum made to the peptide encoding the carboxy terminus decapeptide of transducin, recognized both 40–41 and 43 kDa proteins. It had been previously shown that AS/6 does not recognize α_o but does recognize α_i proteins [11]. These data indicate that both the 43 kDa and 40–41 kDa proteins belong to the α_i-subtype. The 43 kDa protein also appears to have an N-terminus region similar to α_i pro-

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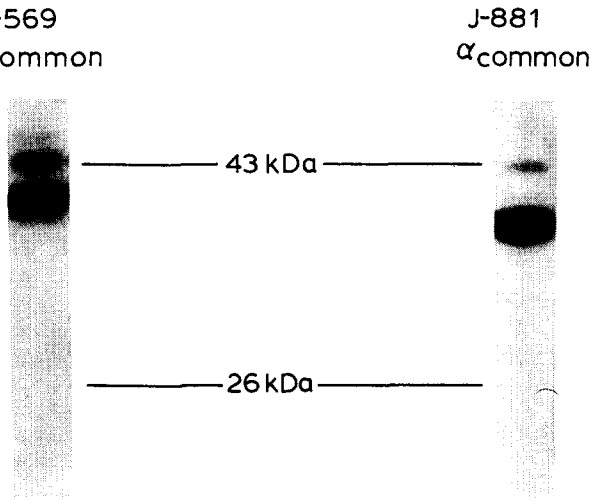

Fig.1. Detection of the 43 kDa protein by immunoblotting of purified human erythrocyte G-proteins with G_α common specific antisera A-569 and J-881. G-proteins (4–6 μg total protein) were resolved electrophoretically on 9% gels and then transferred to nitrocellulose papers. The papers were blocked with ovalbumin and blotted with A-569 (1:1000 dilution) or J-881 (1:500 dilution). Photographs of autoradiograms exposed for 5 days are shown.

teins, since it is recognized by H-660, an N-terminus-specific antiserum that recognizes both α_i and α_o [10] (fig.2). We next tested which, if any, of the α_i-subtype specific antisera would recognize human erythrocyte 40–41 or 43 kDa proteins. We found that all three of the α_i subtypes were present in the human erythrocyte preparations in the 40–41 kDa range. However only the α_{i3}-specific antiserum recognized a 43 kDa protein (fig.3). In this figure we used two preparations of G-proteins made several years apart.

Since the 43 kDa protein is a trace protein and since the antisera used are not very sensitive, a relatively large amount (4–6 μg) of total α-subunits had to be used in the immunoblotting assays. Hence, in the protein concentration range in which the immunoblotting for 43 kDa protein is performed, the autoradiographic intensity of the 40–41 kDa range proteins is not proportional to their protein concentration with the α_i-subtype specific antisera. However, with the α-common antiserum, autoradiographic intensity is proportional to protein concentration since lower protein concentrations can be used. Quantitative immunoblotting with 100–500 ng protein indicates that G_{i2} and G_{i3} comprise about 35% and 50%, respectively, of the total G_i protein in human erythrocytes. G_{i1} was 5% and G_{43} about 10% of the total G_i proteins.

4. DISCUSSION

The data in figs 1–3 indicate that the 43 kDa pertussis toxin substrate is an α_{i3}-like protein. We had previously shown that this protein shifts from 4S to 2S on sucrose density gradient ultracentrifugation upon treatment with GTPγS and Mg^{2+} ions (fig. 7 in [6]). Taken together, all these obervations strongly suggest that the 43 kDa protein is the α-subunit of a heterotrimeric G-protein. The regions defined by the interactions of the various antisera are summarized in fig.4. From this scheme, it can be seen that the N-terminal region, where α_s has several extra amino acids as compared to the α_is and α_o, does not appear to be the region that is extended in α_{43}. This conclusion is based on the observed reactivity of the antiserum H-660, which interacts with this region of α_o and the α_i-family, but not with α_s. While the amino acid sequences shown for these regions may

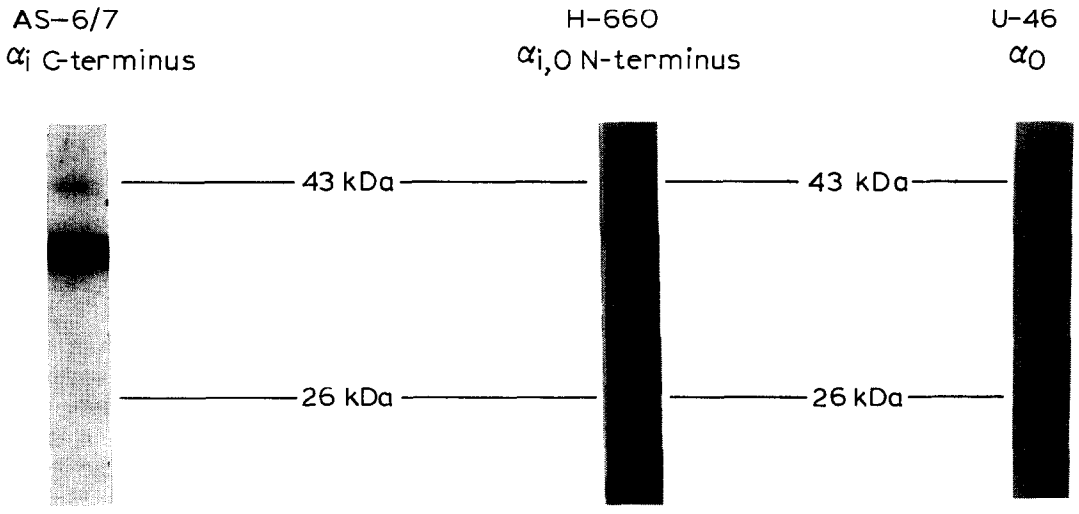

Fig.2. Identification of human erythrocyte pertussis-toxin substrates as G_i proteins. The G-proteins (6 μg total protein) were resolved by electrohoresis on 9% polyacrylamide gels and then transferred to nitrocellulose papers. The antisera were used at the following dilutions: AS/6-7, (1:200); H-660, (1:100); and U-46, (1:1000). The photographs shown were taken from autoradiograms obtained after 2 days exposure for the AS/6-7 blot and seven days exposure for the H-660 and U-46 blots, without the use of intensifying screens. For other details see section 2.

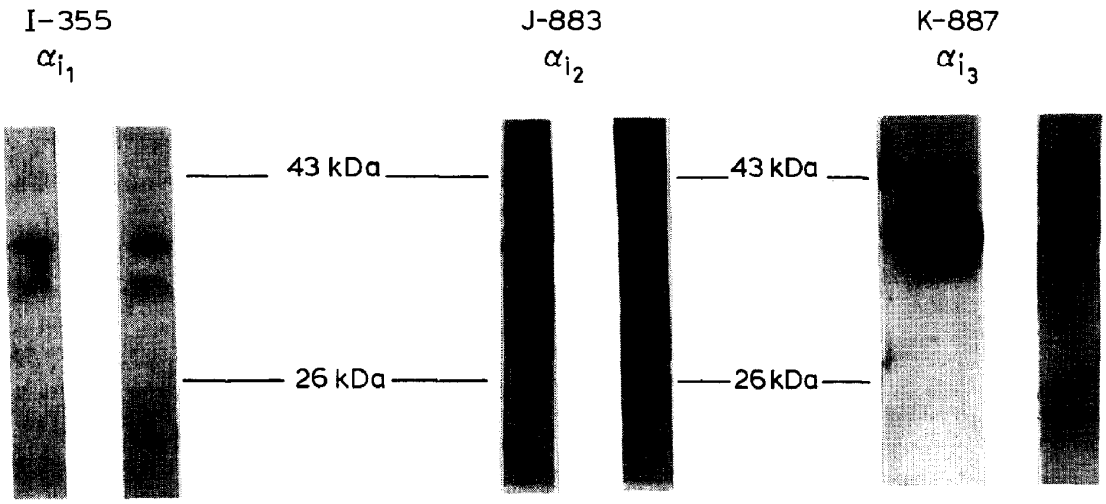

Fig.3. Identification of 43 kDa protein as a G_{i3}-related protein. Two different G-protein preparations (4–6 μg total protein) were blotted against the α_{i1}–, (I-355), α_{i2}-, (J-883), and α_{i3}-specific (K-887) antisera. Photographs were taken from autoradiograms of 5-day exposures without the aid of intensifying screens. For other details see section 2.

not represent the actual sequence of α_{43}, it appears reasonably certain that the sequences of α_{43} are close to what is shown in fig.4, since all the antisera used have been shown not to interact with closely related sequences.

The origin of α_{43} has as yet not been determined. Two possibilities exist. It could be derived from alternative splicing of the G_{i3} gene transcript, or it could be the product of a distinct gene. Recently the genes encoding α_{i1}, α_{i2} and α_{i3} were isolated by screening with a rat α_{i3} cDNA probe. Only a single copy of the α_{i3} gene was found by Southern blotting analysis, and no other α_{i3}-like genes were reported [2]. This would suggest that there does not exist a distinct gene encoding α_{43}. However, at this time, such a possibility cannot be ruled out unequivocably until it is determined that the human genome also contains only one α_{i3} gene. It is possible that α_{43} arises from alternative splicing of the transcript from a single gene in analogy with the four mRNAs encoding the high (52 kDa) and low (42 kDa) molecular weight forms of α_s [12]. These alternatives will have to

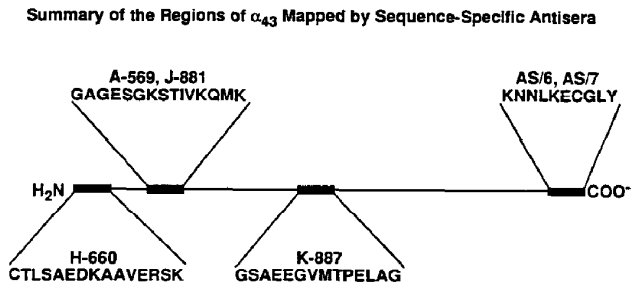

Fig.4. The regions of the α_{43} protein as defined by the interactions with the various sequence-specific antisera. The single letter amino acid code is used. The sequences given represent the sequences derived from the human α_{i3} clone [2].

be addressed experimentally. In spite of the uncertainty about the origin of α_{43}, there is little doubt that G_{43} needs to be added to the growing list of heterotrimeric G-proteins.

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